AFIT/GEE/ENV/96D-12

THE BIODEGRADATION CHARACTERISTICS OF PROPOSED FUEL SYSTEM ICING INHIBITORS (FSII)

THESIS

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THESIS

Presented to the Faculty of the Graduate School of

Engineering

Air Education and Training Command

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Engineering and Environmental Management

Charles E. Meshako, B. S.,

GS-11, DAF

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Member

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Chairman

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Abstract

The biodegradation characteristics of three fuel system icing inhibitors (FSII) were evaluated. FSII are jet fuel additives that partition into water readily and are present in the water drained from storage tank bottoms in concentrations approaching 40%. These concentrations raise concerns as to the disposal and handling of these wastes.

The current FSII, DiEGME was evaluated along with two new candidates, dipropylene glycol and glycerol formal. DiEGME appeared to be moderately but not completely biodegradable. It is likely that much of it would be removed in a wastewater treatment plant. Dipropylene glycol only showed signs of degradation after more than three weeks at which point it degraded moderately well. The third FSII, glycerol formal did not show any signs of biodegradability during the five week period of testing.

Preliminary toxicity and inhibitory tests were carried out for these chemicals at high and low concentrations. DiEGME appeared to be most toxic to microorganisms at high concentrations, dipropylene glycol show moderate toxicity, and glycerol formal showed little. At low concentrations, none of the chemicals appeared to inhibit the activity of microorganisms.

List of Terms

Biological Seed - A culture of microorganisms used as the source of the microbial population in biodegradation testing.

BOD - Biochemical Oxygen Demand - the amount of dissolved oxygen in water required in the aerobic biodegradation of an organic substance under standard test conditions. The numerical subscript denotes the number of days of the test. All oxygen demands in this report are presented in either milligrams of oxygen per liter of pure organic substance or grams of oxygen per gram of organic substance depending upon their magnitude.

COD - Chemical Oxygen Demand - the amount of dissolved oxygen in water required in the oxidation of an organic substance by a strong oxidizing agent under acidic conditions.

ThOD - Theoretical Oxygen Demand - the theoretical amount of dissolved oxygen in water required to completely oxidize an organic substance.

POTW - Publicly Owned Treatment Works - A term used for wastewater treatment facilities that are often operated by a local municipality.

WWTP - Waste Water Treatment Plant

THE BIODEGRADATION CHARACTERISTICS OF PROPOSED FUEL SYSTEM ICING INHIBITORS (FSII)

I. Introduction

Background

The draining of "tank bottoms" from jet fuel tanks is part of the armed forces' daily routine. These tank bottoms consist of water, the soluble components of jet fuel, and a fuel additive known as fuel system icing inhibitor (FSII). As the FSIIs are extremely hydrophilic, they partition to the tank bottoms in concentrations upwards of 40% (Grosjean, 1996A). The presence of these chemicals at such a high concentrations makes the disposal of these bottoms problematic. Concerns over the health and environmental impacts of these wastes have prevented the disposal of tank bottoms into sewage systems serving some Air Force installations. At these sites, the tank bottoms are currently handled and disposed of as hazardous waste (Day, 1996).

A joint effort by the Naval Air Warfare Center and the Air Force's Wright Labs is underway to identify novel chemicals for use as FSII. Preliminary selection was accomplished using quantitative structural activity relationship (QSAR) software. These candidates were then screened for their ability to inhibit ice crystal formation in fuel, their fuel/water partitioning behavior and their stability in jet fuel.

Research Problem

Little information is available on the biodegradation of the current and proposed FSIIs. This information is essential in evaluating the environmental fate and impact of these chemicals.

Research Objectives

The purpose of this research was to develop an understanding of the biodegradation properties of the current and proposed icing inhibitors. Studying the biodegradation characteristics and the acute microbial toxicity will provide information on how these chemicals might behave in a wastewater treatment facility.

Scope

Closed-bottle biodegradation testing included standardized 5-day biochemical oxygen demand (BOD_5) tests, acclimated seed BOD_5 tests, an inhibitory BOD_5 test, and 20-day BOD tests. Respirometric testing was used to augment these tests. Microbial toxicity was evaluated at high FSII concentrations using a qualitative agar diffusion test. Careful design and interpretation of these tests provided basic, reproducible data on the biodegradation characteristics of the FSII.

Limitations

The limitations of this research are those of any laboratory evaluation of a field scale phenomena: extrapolation from these tests to a full scale wastewater treatment plant

(WWTP) should be done with caution. WWTPs are high energy systems which are highly aerated and optimized for microbial growth (Pajak, 1977).

Organization of the Research Report

This report begins with a literature review to provide a background on respirometric biodegradation testing, fuel system icing inhibitors, and the impacts of industrial wastes on biological treatment processes. The methodology used to conduct the experiments is presented in Chapter 3. The results and analysis of the experiments are provided in Chapter 4. Chapter 5 presents the conclusions and recommendations developed from this research.

II. Literature Review

This chapter reviews the literature on the three major areas of this research. Closed-bottle biodegradation tests and their evaluation are discussed in the first section. The second section describes the fuel system icing inhibitors and tank bottom wastes, and the last section discusses the impact of industrial wastes on biological treatment processes.

Biodegradation Testing

The fate of a substance in the environment is one of the most important factors in evaluating its impact on human health and the environment as a whole. The means by which chemicals 'disappear' are frequently broken into the separate categories of biodegradation, photochemical degradation, and chemical degradation. Of these three processes, biodegradation of fuel system icing inhibitors is likely to be the most significant based on their chemical properties as well as "the most desirable because it generally results in complete mineralized end-products" (Howard et al., 1989; Howard et al., 1975). The most direct means of determining the degradation and fate of any substance is to measure the changes in its mass in a controlled experiment. However, these direct measurements are often not practical and an indirect means of testing often must be used. One of the oldest and most effective methods of indirectly measuring the biodegradation of a substance is measuring the consumption of oxygen (Howard et al., 1975). As microorganisms aerobically metabolize an organic chemical, they consume oxygen and produce water, carbon dioxide, biomass, and intermediates of the degradation

process (smaller organic chemicals). As it is very difficult to quantify any of the products of these reactions, the most practical solution is to measure the amount of oxygen which is consumed along with the organic chemical.

Biochemical Oxygen Demand (BOD). Biochemical Oxygen Demand represents the amount of dissolved oxygen required to biodegrade an organic substance under specific standard test conditions (WQA, 1996). It is widely used to measure the amount of organic pollution in wastewater and streams. The standard method of determining BOD is a five day closed bottle test, commonly denoted as BOD_5. The version used in this project is defined in the American Public Health Association's *Supplement to the Standard Methods for the Testing and Evaluation of Water and Wastewater, 18th Edition* (APHA, 1992). The test consists of incubating a sealed bottle containing purified water, the test chemical, and a source of microorganisms (biological seed) for five days and measuring the change in dissolved oxygen. It is widely used in the standardized evaluations of wastewater treatment plants, industrial wastewater permitting, and surface water quality evaluation. The initial five day length of the test was chosen as this is the typical retention time of water in England's rivers before they reach the ocean (Pitter and Chudoba, 1990).

Several variations of the 5-day BOD test have been developed by researchers. Some of these variations include lengthening the time of the test, taking measurements more frequently, altering the biological seed, and inhibiting or correcting for denitrifying bacteria. Many of these variations are documented in a survey of degradation techniques done for the US EPA (Howard et al., 1975).

10-Day BOD Tests. A simple lengthening of the BOD test to 10 days was performed by Mills and Stack in 1954 as cited in Howard's review (Howard et al., 1975). Mills and Stack justified this lengthening after they observed a one to two day lag period in oxygen uptake for synthetic chemicals even when they used acclimated seed.

Long-term BOD test. Howard cites experiments by Elmore in which the length of the test was extended and repeated measurements of DO were taken (Howard et al., 1975). The test was designed for experiments where "(a) the compound requires long acclimation periods, (b) the compound requires long periods for complete degradation, or (c) higher concentrations of the test chemical are used" (Howard et al., 1975). The unique aspects to this test were the employment of a very large reservoir bottle (several liters), multiple measurements, and reaeration of the bottles as necessary.

20-Day BOD Test. Although not officially sanctioned by APHA, 20-day BOD tests have been used by industry for several years and the European Community has a similar twenty day closed bottle biodegradation test (Waggy et al., 1994). Pitter explains that under BOD test conditions "the oxidation of newly produced reserve substances and proteins in the cells lasts for 10 to 20 days" (Pitter et al., 1990). This implies that 20 days is probably the longest a BOD test can be reliably carried out.

Ultimate BOD Test. A modern adaptation of Elmore's test appears in the *Supplement to the 18th Edition of Standard Methods* in the form of a proposed ultimate BOD (UBOD) test (APHA, 1992). Like Elmore's procedure, a reservoir bottle is recommended and reaeration is permitted, but several significant differences appear. The test recommends, but does not require, that two liter sample bottles be used. The

reservoir bottle is used to supply water for topping the sample bottles' water seals between measurements and as a source from which to take samples for nitrate testing.

Acclimated Seed Techniques. As microorganisms often require a period of time to adjust to the presence of a new synthetic chemical, researchers will sometimes attempt to acclimate a culture of microorganisms to a substance prior to a test. This can be accomplished in a laboratory environment by continuously aerating a sample of sewage bacteria and adding the test chemical, minerals, and a secondary source of food such as yeast extract (Howard et al., 1975; Bridie et al., 1979B; Pitter et al., 1990).

Nitrification. The oxygen demand exerted by the nitrification of ammonia to nitrite and eventually to nitrate sometimes needs to be addressed in biochemical oxygen demand testing. This demand is usually not a problem in 5-day tests as it tends to be negligible if proper care is taken. As the nitrifying bacteria grow very slowly, minimizing the initial population of such microorganisms in a test is a practical method of limiting their influence in 5-day tests. Obtaining distilled water which has not been stored for very long and obtaining seed from raw sewage rather than activated sludge limits the influence of these nitrifiers in short tests. Other methods to account for nitrification include inhibiting nitrification through the addition of 2-chloro-6-(trichloro methyl) pyridine, as called for in the carbonaceous BOD (CBOD) test, or by simply measuring the accumulation of nitrate and subtracting out the nitrogenous BOD (NBOD) (APHA, 1992).

Automatic Respirometry. As some of the early automatic respirometry experiments were titled "An Improved Apparatus for BOD Testing" and "Determining BOD with Continuous Recording of Oxygen Uptake", it is not hard to see the connection

between these methods. (Howard et al., 1975). Essentially the second title captures the concept of respirometry oxygen uptake is automatically measured continuously (or at least more frequently than would be possible manually). Closed-loop respirometry is a new technique in which measurements and reaerations are controlled using a digital computer (Columbus Instruments, 1994). As the system is 'closed', this technique is less sensitive to aberrations which plague electrolytic respirometry due to such things as fluctuations in barometric pressure. The only apparent drawbacks to closed-loop respirometry are that the measurements are not continuous and the apparatus has a high part count. Even so, the relative ease of operation provides promise that closed-loop respirometry may prove to be the tool of choice when relatively long-term respirometric testing is necessary.

Summary of Respirometric Biodegradation Tests. To summarize the biodegradation testing section, the measurement of oxygen uptake is one of the oldest and most practical means of assessing aerobic biodegradation. The strength of the BOD_5 testing is its standardization and hence, comparability. When the determination of actual kinetics of biodegradation of a substance is necessary, automatic respirometry is considered the best tool for relatively short measurements, where long-term testing favors UBOD.

Evaluation of Biodegradation Testing

Interpreting the results from BOD or other biodegradation tests is somewhat of an art. Any laboratory (or field) experiment is dependent on conditions specific to that test, and this is especially true in biodegradation experiments. A well designed experiment will either strive to reproduce conditions that closely approximate realistic conditions or

attempt to conform to a standardized test from which the results from other such tests can be compared. The BOD test clearly falls into the latter category.

Theoretical Oxygen Demand. A method of interpreting BOD_5 is to compare it to the theoretical total amount of oxygen required to oxidize the test substance. If the chemical formula of the test substance is known, the total amount of oxygen required can be determined theoretically if the chemical formula of the test substance is known using the following equation:

$$ThOD := \frac{\left(n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4}\cdot c\right)\cdot 32}{(n\cdot 12.011 + a + 15.99b + 14.007c)}$$

where
n = the number of carbon atoms per molecule,
a = the number of hydrogen atoms per molecule,
b = the number of oxygen atoms per molecule, and
c = the number of nitrogen atoms per molecule.

This equation is a rearrangement of an equation that appears *in Chemistry for Environmental Engineers* (Sawyer et al., 1994).

However one rarely knows the exact chemical formula of mixed wastewater. In such a case, the oxygen required to oxidize a substance can be determined by using a standardized test in which the chemical is oxidized by a strong oxidizer in an acid bath. The results of this test are known as the chemical oxygen demand (COD) of the substance (APHA, 1992). COD is typically just slightly less than ThOD. The ratio of BOD_5/COD or BOD_5/ThOD can both provide a rough estimation of biodegradability.

Lyman's BOD_5/COD Classification. Lyman has established a simple classification scheme for biodegradability using the BOD_5/COD ratio (Lyman et al., 1990). Lyman classified chemicals with a BOD_5/COD ratio of less than 1% as "relatively

undegradable." Chemicals with a ratio between 1 and 10% were called "moderately degradable." Chemicals with ratios greater than 10% were considered "relatively degradable."

Although the method of using a ratio of two oxygen demands does provide a simple classification method using standardized values, it only provides as much information as one can expect to get from a single "snap shot" in time. Much more valuable information can be obtained by evaluating the kinetics of degradation using data collected across several points in time. This information can be presented by plotting the results of a long-term BOD or respirometry tests vs. time. This information can either be viewed qualitatively or matched to theoretical models.

BOD Tests as Toxicity Screening Tests. Toxicity to microorganisms can be evaluated by comparing the amount of oxygen consumed in a BOD_5 test over a range of concentrations. A decrease in oxygen consumption with an increase in substrate concentration provides evidence of toxicity (Pitter and Chudoba, 1990; Wetzel and Murphy, 1991). The obvious limitation in this type of test is that the range of the BOD_5 test is not very large and only a small range of low concentrations can be screened.

Fuel System Icing Inhibitors (FSII)

History of Fuel System Icing Inhibitors. Research into FSIIs commenced after the crash of a B-52 in 1958 was attributed to the clogging of screens and filters. These filters appeared to be clogged by either "some form of fuel contaminant or ice formation" (Finefrock, 1966). The water which is dissolved in fuel can freeze into ice crystals which may block fuel filters and/or jam controls. The civilian approach to this problem is

to install heaters in the fuel filters. However, concerns of weight and bulk prevented military aircraft from doing the same (Goodger, 1995).

The solution of the military was to use a fuel additive originally called an anti-icing additive (AIA), but now known as a fuel system icing inhibitor (FSII) (Finefrock, 1966). Performance characteristics required that it be very hydrophilic, highly stable in fuel, and able to effectively depress the freezing point of water. By 1961, ethylene glycol monomethyl ether (EGME), $CH_3OCH_2CH_2OH$, was added to jet fuel at the point of manufacture (Finefrock, 1966). EGME can be viewed as the smallest possible oxyethylated alkyl ether or ethyoxylated alcohol as it is composed of one oxyethylene (OE) chain and a methyl group. EGME and 13 other industrial solvents recently came under EPA scrutiny. The US EPA's 14 August 1996 proposed rule decided not to list EGME as a listed RCRA hazardous waste because "a lack of data indicating widespread use" (DER, 1996). Consistent with QSAR studies that show that increasing the OE chain length decreases the toxicity in crustaceans, EGME has been phased out and replaced with the less toxic diethylene glycol monomethyl ether (DiEGME), $CH_3OCH_2CH_2OCH_2CH_2OH$ (Schuurman, 1990; Bridie et al., 1979A). The Navy was the first to adopt DiEGME for use in JP-5 due to its to its higher flash point (Dow Chemical, 1989). The switch to higher flash point fuels by the Air Force coupled with concerns of toxicity prompted the Air Force to follow suit and begin using DiEGME in JP-8 in 1993 (NAVAIRWARCENACDIVTRENTON, 1996).

Water contamination of fuel occurs when humid air replaces the fuel pumped out of storage tanks. The amount of water absorbed from make-up air under typical conditions has been estimated to be approximately 1 gallon of water for every 20 to 30

thousand gallons of jet fuel pumped (Grosjean, 1996A). Water can also be introduced to uncovered floating roof storage tanks by rain fall. These floating-roofs are common at Air Force installations in the western U.S. where rainfall is less common, but single rainfall events can be quite intense. This can result in large volumes of water being introduced into the fuel tanks in very short periods of time (Day, 1996). A third process by which water can be introduced into fuel is during transport. This is especially problematic at installations that receive their fuel shipments by barge or underground pipeline (Day, 1996).

The water that comes into contact with the fuel is either dissolved by the fuel or remains a separate phase and sinks to the bottom of the fuel storage tank. Some of the dissolved water falls out of the fuel as it cools. This water accumulates on the bottom of the tanks and is termed "tank bottoms". These tank bottoms are essentially a cocktail of water, FSII, and the soluble components of jet fuel. Reports of concentration of FSII in the tank bottoms range from 20% to nearly 50% (Grosjean, 1996A; Goodger, 1981). These higher concentrations of FSII act as an effective biocide (Finefrock, 1966). The concentration of FSII in tank bottoms depends on the type of FSII, the concentration of FSII in the fuel, and how the water came to rest on the bottom of the tank. The typical disposal of fuel storage tank bottoms has been through the municipal sewage system. However, as BOD and COD of these tank bottoms is quite high and can violate many sewage discharge permit limits if it is released directly into the municipal sewage system, a very slow release or pretreatment is often required. Some POTWs have refused to accept these FSII laced tank bottoms and disposal is handled by draining the tank bottoms into 55 gallon drums to be disposed of as hazardous waste (Day, 1996). FSII also enter

the environment when aircraft lay-over at a base overnight. Prior to take-off the next morning, the tanks bottoms of the aircraft fuel tanks are drained, typically directly on to the apron.

The FSII Joint Initiative. The possible environmental impact and potential future regulation of ethylene glycol ethers by regulators, have led the Naval Air Warfare Center (NAWC) and the Air Force's Wright Laboratory to launch an initiative to find new chemicals which can be used to replace DiEGME. This research has taken the form of identifying potential FSIIs using QSAR molecular modeling software. The two basic approaches in the search for new FSIIs have been to alter existing icing inhibitors into more benign substances, or to alter already benign substances such as biologically based sugars to improve their deicing characteristics (Trohalaki and Pachter, 1996; Mushrush et al., 1996). Tests of their effectiveness as FSIIs were conducted on a bench scale experimental rig at NAWAC (NAVAIRWARCENACDIVTRENTON, 1996). Tests of the fuel water partitioning characteristics were conducted at WPAFB (Grosjean, 1996B). The most promising candidates became the subject of this research project.

Proposed FSIIs. The critical performance characteristics of FSII include extreme hydrophilicity, stability in fuel, and the ability to depress the freezing point of water. The three selected chemicals share these characteristics as well as the fact that they are all ethers with at least one alcohol group.

TABLE 1
PHYSICAL PROPERTIES OF FSIIS

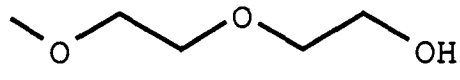
Chemical	Molecular Weight	Specific Gravity	Vapor Pressure	Water/Fuel Partition Ratio	ThOD (g/g)
DiEGME	120.15	1.054[1]	0.08 mm Hg @ 20 C[1]	480[2]	1.73
Glycerol Formal	104.11	1.203		570[2]	1.38
Dipropylene Glycol	134.18	1.0224[3]	1 mm Hg @ 73.8 C[3]	> 600[2]	1.91

Sources: 1) Dow Chemical, 1989 2) Grosjean, 1996B. 3) NTP, 1996

The biodegradation of ethers has "not yet been studied in detail" (Pitter and Chudoba, 1990). To emphasize this point one can look to the following proposals for the bacterial metabolism of ethers. Kawai states that "EG monomethyl ether and DEG monoethyl ether were shown to be oxidized at their terminal alcohol" (Kawai, 1995). The CAS abstract of Kravetz's "Ultimate Biodegradation of an Alcohol Ethoxylate and a Nonphenol Ethoxylate under Realistic Conditions" identifies the "cleavage of the POE (polyoxyethylene) from the alkyl chain as the initial step" (CAS, 1982). Tessier proposes a scheme in which the enzyme extracted from an *Acinetobacter* strain attacks the oxygen atom between the oxyethylene chains (Tessier, 1983). Although these variations might be explained by the potential that each test was using different microbes, they do little to clarify the issue.

FSII Summaries

Diethylene Glycol Monomethyl Ether (DiEGME) [CAS Registry Number 111-77-3]



Howard's *Handbook of Environmental Fate and Exposure Data* cites a US EPA survey in which DiEGME is listed as a drinking water contaminant in Pomona,

Enscondido, Lake Tahoe and Orange Co., CA; Dallas, TX; Washington, DC; Cincinnati, OH; Philadelphia, PA; New Orleans, LA; Ottumwa, IA; and Seattle, WA (Howard et al., 1989).

DiEGME is not a characteristic or listed hazardous waste according to RCRA 40 CFR 261. However, Howard notes that DiEGME is regulated by SARA Section 313 listing and the TSCA Chemical Inventory June 1990 (Howard et al., 1989).

BOD test results for DiEGME have been published by both Shell Research and Dow Chemical. Bridie's testing for Shell followed the standard 5-day APHA testing procedure and is presented in Table 2 (Bridie et al., 1979B).

TABLE 2
BRIDIE'S TEST RESULTS FOR BOD AND COD OF DIEGME

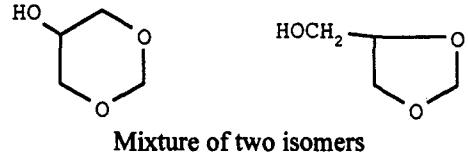
	ThOD (g/g)	BOD_5 (g/g)	BOD_5/ThOD	COD (g/g)	COD/ThOD
DiEGME	1.73	0.12	7%	1.71	99%

Dow Chemical conducted 20-d BOD tests. The results are presented in Table 3. Dow's results differed substantially from Bridie's tests that little or no oxygen seemed to be consumed in the first five days (Dow Chemical, 1989).

TABLE 3
DOW CHEMICALS 20-DAY BOD TEST RESULTS FOR DIEGME

	Day 5 (BOD/ThOD)	Day 10 (BOD/ThOD)	Day 10 (BOD/ThOD)
DiEGME	0%	21%	66%

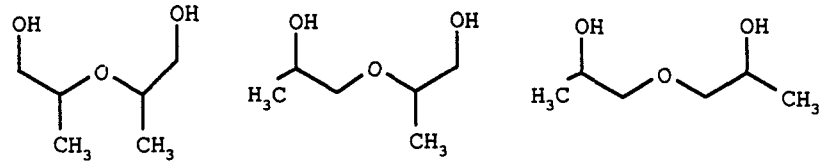
Glycerol Formal (CAS Registry Number 86687-05-0)



Mixture of two isomers

Glycerol Formal is a commercially available substance that is currently used primarily as a pharmaceutical solvent in veterinary science. It may possess similarities to other cyclic ethers such as dioxane and tetrahydrofuran whose biodegradation has required the isolation of special strains of microorganisms (Bernhardt et al., 1991; Roy, 1994).

Dipropylene Glycol (DPG) (CAS Registry Number 25265-71-8)



mixture of isomers

The studies of the biodegradation of dipropylene glycol are limited to preliminary screening tests such as Bridie's BOD tests (Bridie et al., 1979B; Howard et al., 1989) and preliminary biotechnology tests using isolated strains (Kawai, 1995). Bridie's BOD_5 test results are presented in Table 4. Kawai's testing demonstrated increases in the turbidity of flasks that contained isolated strains of dipropylene glycol utilizing microorganisms.

TABLE 4
BRIDIE'S BOD AND COD TEST RESULTS FOR DPG

	ThOD (g/g)	BOD (g/g)	BOD/ThOD	COD (g/g)	COD/ThOD
DPG	1.91	0.09	5%	1.84	97%

Howard cites a USEPA survey in which DPG is listed as a drinking water contaminant in Pomona, Enscondido, Lake Tahoe and Orange Co., CA; Dallas, TX; Washington, DC; Cincinnati, OH; Philadelphia, PA; Miami, FL; New Orleans, LA; Ottumwa, IA; and Seattle, WA (Howard et al., 1989).

POTW Impacts

Edward Wetzel compiled several studies conducted for the U.S. EPA on POTW interferences by industrial wastes in a single volume titled "Treating Industrial Waste Interferences at Publicly-Owned Treatment Works" (Wetzel and Murphy, 1991). This work summarizes the problem of POTW interferences and includes the results of 29 case studies of POTWs that had reported upsets due to industrial wastes. These upsets tended to result from intermittent releases. The source of these releases tended to be wastes from infrequent industrial processes or illegal "midnight dumping". In addition to the high concentrations and toxicities found in these types of releases, the intermittence of these events ensured that "biological populations are...not acclimated to either the specific compounds or concentration levels." (Wetzel and Murphy, 1991).

Typical BOD influent limits for POTWs run from 200 to 300 mg/L. Industries in Union Beach, NJ have industrial waste permits with maximum allowable concentration limits for BOD(500). COD(1500) and TSS(500). (Wetzel and Murphy, 1991).

As cited by Bishop, Helfgott provides suggested criteria for influent for a sewage treatment plant. His suggested criteria for industrial wastewater are listed below:

1) $BOD_5 < 150 - 650$ mg/L
2) $BOD_{20} <$ Twice the BOD_5
3) ThOD / UBOD < 1.1
4) (ThOD + TN) / UBOD < 1.1 (for wastes with nitrogenous components)

where:

UBOD = ultimate biochemical oxygen demand,
ThOD = theoretical oxygen demand, and
TN = total oxidizible nitrogen (Bishop, 1987).

Although the EPA has not adopted these criteria to date, these criteria do provide a more thorough description of a waste's impact on receiving waters than the classical BOD and COD parameters alone.

Andrew Pajak compiled the results of several different biodegradation studies in an effort to evaluate the impact of "hazardous material spills on biological treatment processes" (Pajak, 1977). This study warns that comparisons of laboratory tests to actual full scale treatment plants is difficult as several factors such as "the concentration of the chemical", "the degree of acclimation", "the aeration rate", "type of treatment", "concentration of mixed liquor suspended solids", "temperature", "detention time", and the "presence of other materials" can dramatically alter the results of the study. This study also categorizes potential biological interferences of hazardous waste spills including

- direct toxicity to organisms,
- inhibition of biological processes,
- exertion of BOD after treatment (e. g. in receiving stream),
- high effluent COD caused by refractory material,
- disruption of sludge treatment, and
- high oxygen demands resulting in a poor bioreaction environment.

These efforts provide some simple guidelines that address the complex issue of whether a particular waste is suitable for disposal in a wastewater treatment plant. None claim to be the 'last word' on this subject as extrapolation of small scale results to full scale sewage plants is very difficult.

III. Methodology

Five Day Biochemical Oxygen Demand (BOD_5) Test

Introduction. The BOD_5 tests conducted in this research were based on the *Supplement to the Standard Methods for the Examination of Water and Wastewater, 18th Edition* (APHA, 1992). These closed bottle tests combine the target chemical with buffered dilution water, biological seed, and are incubated in the dark for five-days at 20°C. The dissolved oxygen (DO) levels are measured at the beginning and the end of the incubation using a DO electrode probe. The measured difference in dissolved oxygen is due to the consumption of oxygen by the microbial utilization of the test compound and the background metabolism associated with the biological seed. The BOD is calculated by subtracting the oxygen consumption of the biological seed from the total oxygen consumption and dividing the result by concentration of the seed in the bottle.

BOD Test Specifics. The bottles in all of the BOD tests were heavy glass 300ml bottles from Wheaton with glass stoppers and flared mouths. The bottles were completely filled so that no head space exists (which would provide an unwanted source of oxygen). After the stopper was inserted, the bowl formed by the flared mouth was filled with water and a small disposable plastic cup was inverted over the top of the bottle to minimize the evaporation of the water seal during incubation. The bottles were all stored in a dark incubator set to maintain a temperature of 20°C ± 1°C. Quality controls and replicates that were conducted are presented in Table 5.

TABLE 5
BOD QUALITY CONTROLS/SAMPLES FOR EACH TEST

Sample Type	Replicates	Vol. of Sample	Goals/Standards
Dilution Water Check	2	2 ml	> 0.2 mg/L required
Seed Controls	3	2-3, 5-7, 6-9 ml	0.6 to 1.0 mg/L desired
Glucose/Glutamic Acid Solution Check	2	6 ml	BOD of 198.5 mg/L +/- 30.5 mg/L
Test Samples	3	-	At least 2 mg/L depleted and at least 1 mg/L remaining

Dilution Water Check. The dilution water quality test ensures that the presence of organics or nitrifying bacteria in the water is minimal. Either could have the potential to interfere with the testing by exerting an oxygen demand which was related to the dilution water rather than the biological seed and the target substance. Dilution water was either 18 MΩ reagent grade water from a Millipore filter system or commercial distilled water purchased at Meijer supermarket. This water is cooled to 20°C prior to testing. A buffer solution of phosphate, calcium chloride, magnesium sulfate, and sodium sulfate prepackaged by Hach Chemical Company was added to the dilution immediately prior to aeration to provide nutrients and stabilize the pH. The pH of the dilution water was confirmed to be between 7.0 and 7.2 prior to testing.

Seed Controls. The seed control test provides a measure of the oxygen demand of the organics present in the biological seed as well as the normal oxygen consumption of the microorganisms. The 0.6 to 1.0 mg/L depletion goal ensures that seed activity is high enough to be effective but low enough so that it does not significantly limit the range of the test. Three different concentrations of seed are used for each control. The lowest concentration corresponds to the level found in test samples. The two higher

concentrations ensure that oxygen depletion is large enough to provide readings which are significantly greater than the natural variability (i.e. higher signal to noise ratio).

The BOD of the seed is calculated for the three concentrations and the average is used. The biological seed for all but one of the successful tests was the supernatant of raw wastewater from the influent of the Fairborn Water Reclamation Center, Fairborn, Ohio. This wastewater was collected by plant personnel and settled for 24 hours.

Glucose/Glutamic Acid Check. The glucose/glutamic acid solution check provides a standard by which inter-laboratory and test-to-test consistency can be determined. The test solution was prepared per *Standard Methods* using 75 mg of glucose (granulated form) and 75 mg of glutamic acid (powder form) in 500 ml of distilled water. This glucose/glutamic solution should have a BOD of 198.5 mg/L ± 30 mg/L. Possible variations in this check include variations in seed health, the presence of toxins or inhibitors in the dilution water (e.g. copper from the distillation process), or improper incubation.

Test Samples. The test samples were formulated using buffered dilution water. BOD testing of the FSII compounds were performed in triplicate for the BOD_5 and six replicates for the long-term BOD.

Test Set-Up Procedure. BOD test set-up is described:

1) Procure 9 to 18 liters of distilled water and store overnight at 20°C.
2) Place distilled water in thoroughly cleaned and rinsed carboy.
3) Mix in prepackaged buffer solution and aerate for approximately 30 minutes.
4) Prepare glucose/glutamic acid solution and stir.
5) Prepare test chemical solution and stir.
6) Calibrate DO probes after removing condensed water from probes.
7) Prepare dilution water samples and take initial DO measurements.

8) Mix biological seed into carboy at a rate of 2 to 3 milliliters per each 300 ml volume remaining in the carboy. Concentration of seed judged from turbidity of seed and rainfall conditions of previous day.
9) Construct first seed control using seed/buffered dilution water mixture straight from carboy.
10) Construct second and third seed controls by pipetting additional biological seed into the bottles directly.
11) Construct two glucose/glutamic acid check controls by pipetting six milliliters of glucose/glutamic acid solution into each bottle.
12) Construct test compound samples in triplicate (BOD_5) or six replicates (UBOD) by pipetting test compound solution into bottle.
13) Measure DO in each bottle, seal bottles and place into incubator (20°C) for 5 days being sure to maintain water seals through period checking and refilling.
14) Remove bottles after 5-days and measure DO in each bottle.

BOD Calculation. The following calculations subtract the oxygen demand of the biological seed from the oxygen demand of the samples and divides the difference by the concentration of the test chemical.

$$\text{BOD, mg/L} = \frac{\left(D_1 - D_2\right) - \left(B_1 - B_2\right)\cdot f}{P}$$

where:
D_1 = DO of diluted sample immediately after preparation, mg/L,
D_2 = DO of diluted sample after 5 days incubation, mg/L,
P = decimal volumetric fraction of sample used,
B_1 = DO of seed control immediately after preparation, mg/L,
B_2 = DO of seed control after 5 days incubation, mg/L, and
f = ratio of seed in sample to seed in control (APHA, 1992).

Although this calculation provided in *Standard Methods* works well for single samples, the calculations performed in this work were rearranged so that a more rigorous error propagation analysis could be more easily accomplished, as described below.

The first step taken was to calculate the BOD of each of the seed controls using the following equation:

$$\text{BODs, mg/L} = \frac{B_1 - B_2}{P_s}$$

where

P_s = decimal volumetric fraction of seed in a given seed control.

The average and standard deviation of these results is calculated. These two values are used to calculate the "Seed DO" for seed at the concentrations in the test bottles.

"Seed DO" = Ave. Seed BOD * Seed Vol. Conc. in Samples

Next the raw BOD of the sample is calculated ignoring the contribution of the seed as follows:

$$\text{Raw BOD, mg/L} = \frac{D_1 - D_2}{P}$$

The average and standard deviations of these values then are calculated. Next the contribution of the "Seed DO" to this BOD determined:

$$\text{Seed Portion of Raw BOD, mg/L} = \frac{\text{Seed DO}}{P}$$

Finally the average of the Actual BOD is calculated as follows:

Actual BOD, mg/L = Raw BOD - Seed Portion of Raw BOD

This "Actual BOD" is the same as APHA's BOD. This method requires several steps, however it simplifies the calculation of the standard deviation associated with the seed controls and the standard deviation associated with samples themselves.

BOD_5 Test Scheduling. The concentrations covered in each test were chosen such that the first test would cover a broad range of concentrations with the hope that one of the tests would fall in the successful range. For DiEGME, this was followed by additionally testing over a narrower range of concentrations centered around the BOD_5 tests limits. These are presented in Table 6.

TABLE 6
BOD_5 TEST SCHEDULE

Compound	Concentrations (ppm)	Dates Performed
DiEGME	18, 35, 70, and 140	7/18 - 7/23
DiEGME	18, 35, 70, 140, and 1,400	7/26 - 7/31
Glycerol Formal	4, 8, 20, 40, 80, and 201	8/06 - 8/11
DiEGME	14, 28, and 42	8/13 - 8/18
Glycerol Formal	201, 602, and 1003	8/13 - 8/18
DPG	14, 27, 68,136, and 273	8/23 - 8/28
DPG	341, 682, 1704, 3408, and 6816	8/30 - 9/04

Acclimated Seed Tests

These tests are identical to standard BOD_5 tests with the exception that the biological seed is acclimated to the test compound. This acclimation was accomplished by continuously aerating the sludge in 1000 ml Erlenmeyer flasks and feeding the sludge small doses of the test compound every other day (dosing sludge to approximately 50 ppm per feeding). Nutrients in the form of BOD buffer solution were added twice a week. If the health of the seed was in question, a yeast extract was supplemented in concentrations of 25 ppm. The microorganisms were acclimated for approximately 2 to 3

weeks before testing was attempted. Prior to testing the seed was not fed for 4 to 7 days to allow for the degradation of the remaining compound whose presence would interfere with the testing by exerting a large BOD. During acclimated seed testing the seed control goal of 0.6 to 1.0 mg/L depletion was relaxed but the glucose/glutamic acid check values were maintained. The schedule of these tests is provided in Table 7.

TABLE 7
ACCLIMATED SEED TEST SCHEDULE

Compound	Concentrations (ppm)	Dates Performed
DiEGME	7 and 14	9/06 - 9/11
Dipropylene Glycol	7 and 14	9/06 - 9/11
Glycerol Formal	35 and 70	9/15 - 9/20

Inhibition Tests

A modified BOD_5 test was conducted in which glucose/glutamic acid was used a secondary substrate. A decrease in the oxygen consumption of the bottles containing the glucose/glutamic acid mixture and the test compound in comparison with the bottles containing only glucose/glutamic acid would indicate inhibition by the test compound.

Long-term BOD Tests

The long-term BOD tests were conducted in accordance with the proposed ultimate BOD guidelines outlined in the *Supplement to Standard Methods, 18th Edition* (APHA, 1992). Two long-term BOD tests were performed. In the first experiment all three FSII were tested and the three seed controls had stepped concentrations like the BOD_5 tests. This proved to be a problem as the nitrifying bacteria exerted a significant oxygen demand using the buffer solution as a substrate. As a result the two stepped seed controls that had a different amount of seed lost their value as the relationship between

the oxygen demand of seed and the oxygen demand made BOD calculations of all three steps difficult to relate. The second experiment tested DiEGME at three different concentrations and used three seed controls that had the same concentration of seed as the samples.

Respirometric Testing

A closed loop respirometer was used to evaluate the oxygen uptake and carbon dioxide production of microorganisms in the presence of the test chemicals. The test was conducted using 1L bottles that contained 800 ml of buffered BOD water and biological seed at a volumetric concentration of 2.5%. These were incubated at 29°C ± 1°C. An important consideration in this test is that the oxygen concentration of the head space of the bottles was measured as opposed to the concentration of the dissolved oxygen of the water. As the incubator was not large enough to hold stirrers for each bottle, the bottles were swirled by hand between readings 6 and 7; 26 and 27; and 56 and 57. The schedule of the respirometer test is proved in Table 8.

TABLE 8
RESPIROMETER TEST SCHEDULE

Substance	Replicates	Volumetric Concentration (ppm vol.)
Control	2	-
Dipropylene Glycol	2	12.5
Glycerol Formal	2	12.5
DiEGME	2	12.5
DiEGME	2	50.0

Agar Diffusion Toxicity Test

A simple, qualitative toxicity test was conducted using microbial cultures on agar in Petri dishes. The test consisted of spreading three drops or approximately 0.1 ml of biological seed across a nutrient agar surface. Next, a small hole was made in the center of the agar surface and approximately 0.05 ml of the test compound was placed in the hole. The test compound spread outward from the center of the indention and will inhibit the growth of microbes until a low enough concentration is reached. At this distance from the center a distinct 'halo' may form. The radius of this 'halo' provides qualitative information on the toxicity of the compound with larger halos being an indication of greater toxicity. The test was performed in triplicate for each compound and in duplicate for the control. This test was similar to the Agar Diffusion Method in Hurst's *Handbook of Environmental Microbiology* (Hurst, 1996).

TABLE 9
LABORATORY EQUIPMENT USED

Type of Equipment	Make	Model	Serial #
BOD Probe 1	Orion	97-08-00	
BOD Meter 1	Orion	920A	
BOD Probe 2	YSI	5905 BOD	
BOD Meter 2	YSI	Model 58	
BOD Incubator 20C	Lab-Line	Ambi-Hi-Low Chamber 3550	596-005
Balance	OHAUS	Analytical Plus AP2500	1132770180
Respirometer	Columbus Instruments	Micro-Oxymax	94274
Incubator 30C	Lab Line	Imperial III	0396-0326

TABLE 10
CHEMICALS USED

Reagent	Supplier
Glycerol Formal	Aldrich
Dipropylene Glycol (Industrial Grade)	Arco
DiEGME	Aldrich
BOD Nutrient Buffer Pillows	HACH
Glucose	HACH
Glutamic Acid	HACH
Microbial Seed (24 hr Settled Raw Sewage)	Fairborn Water Reclamation Center

IV. Results & Analysis

DiEGME BOD_5 Tests

The results of the DiEGME tests are provided in Table 11. The results labeled "Oxygen (O_2) Consumed" are given in milligrams of oxygen per liter of water. The results labeled "BOD_5" are given in grams of oxygen per gram of test chemical. The results from the tests which where completed on 23 July are not included as the seed DO was not within the prescribed limits and the results were highly variable (See Appendix A). The results from 31 July appeared to be very reliable even though the dilution water check exceeded the limits by 0.09 mg/L. These are included but would not be reportable if we strictly adhere to *Standard Methods* criteria.

TABLE 11
BOD_5 DIEGME RESULTS

Test Date	Conc. (ppm)	O_2 Consumed (mg/L)	BOD_5 (g/g)	BOD_5/ThOD (%)
7/31/96	18	3.46 ± 0.77	0.197 ± 0.044	11.4 ± 5.2
	35	3.17 ± 0.24	0.090 ± 0.007	5.2 ± 0.4
	70	6.89 ± 0.10*	> 0.029	> 1.7
	140	6.78*	> 0.014	> 0.83
	1,400	6.62*	> 0.0014	> 0.08
8/18/96	14	3.84 ± 0.52	0.274 ± 0.037	15.8 ± 2.2
	28	3.31 ± 1.02	0.118 ± 0.036	6.8 ± 2.1
	42	3.18 ± 0.29	0.076 ± 0.068	4.4 ± 0.4
09/09/96	7.0	1.87 ± 0.55**	0.266 ± 0.078	15.3 ± 4.5
10/06/96	3.5	1.61 ± 0.46**	0.457 ± 0.130	26.4 ± 7.5
	7.0	1.93 ± 0.21**	0.274 ± 0.030	15.8 ± 1.8
	14	2.91 ± 0.84	0.207 ± 0.060	12.0 ± 3.5

* exceeded range of test (less than 1 mg/L remaining) (results w/o std dev are singles for screening).
** below reportable range of 2 mg/L depleted.

These results indicate that DiEGME is moderately biodegradable and that the BOD_5 values were dependent on the concentration of DiEGME present in each bottle.

FIGURE 1
DIEGME 5-DAY RESULTS

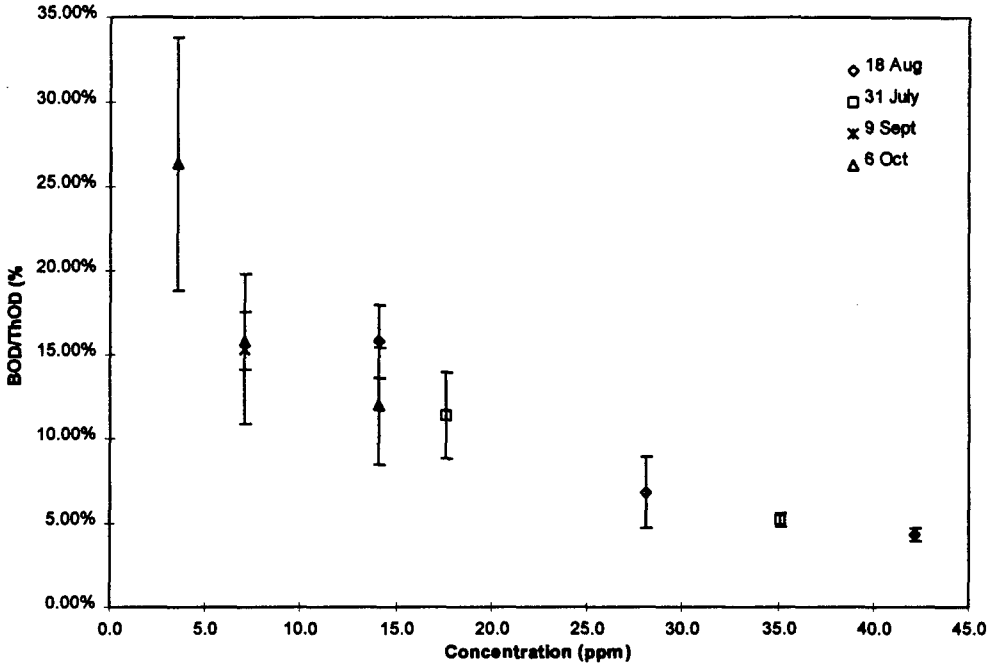
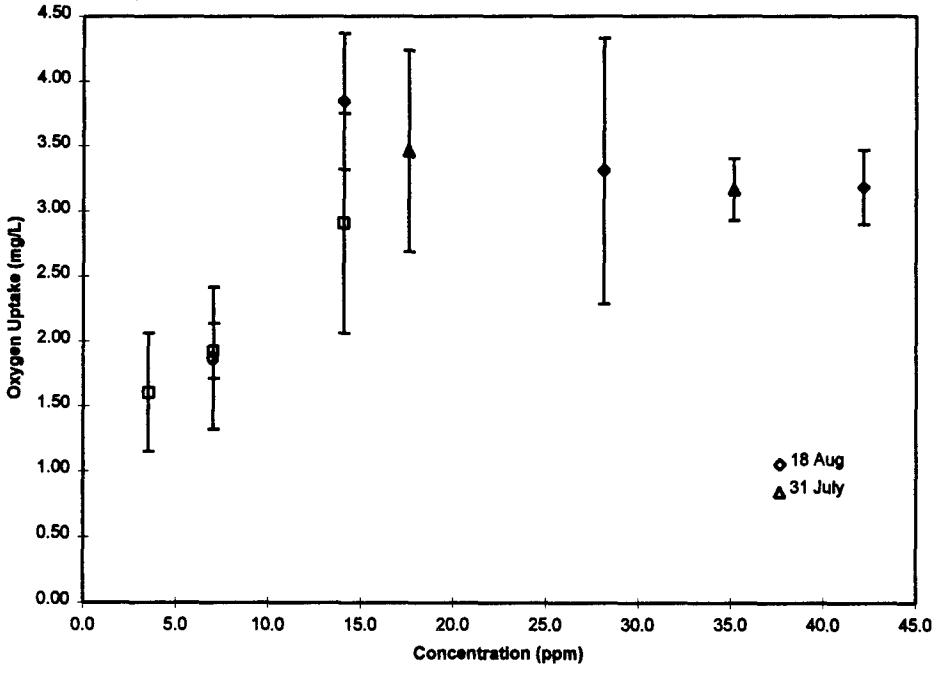


FIGURE 2
5-DAY OXYGEN UPTAKE FOR DIEGME



The BOD/ThOD of DiEGME decreased as the concentration increased (Figure 1).

Further insight into this relationship was seen in a plot of the raw oxygen uptakes (Figure

2). Oxygen uptake appeared to increase with DiEGME concentration from 3.5 to 14 ppm, but leveled off for DiEGME concentrations from 14 to 35 ppm. Oddly the results at 70 ppm and above (not shown) exceeded the range of the test indicating that at least 6.5 mg/L of oxygen was depleted at these concentrations.

A potential explanation for this behavior may be that region below 14 ppm was not limited by the initial number of microorganisms. It may be that the microorganisms that utilize DiEGME are relatively scarce. At concentrations below 14 ppm, enough microorganisms exist so that increases in DiEGME concentrations result in increases in enzymatic activity and oxygen uptake. At concentrations above 14 ppm, any additional activity would require population growth. This stimulation of growth may require a significant increase in concentration. This would explain the seemingly 'flat' region of oxygen consumption.

TABLE 12
REPORTABLE BOD_5 VALUES FOR DIEGME

	Completion Date	Range of Conc. (ppm)	Reportable BOD_5 (g/g)	Reportable BOD_5/ThOD (%)
DiEGME	08/18/96	14, 28, 42	0.16 ± 0.09	8.9 ± 5.4

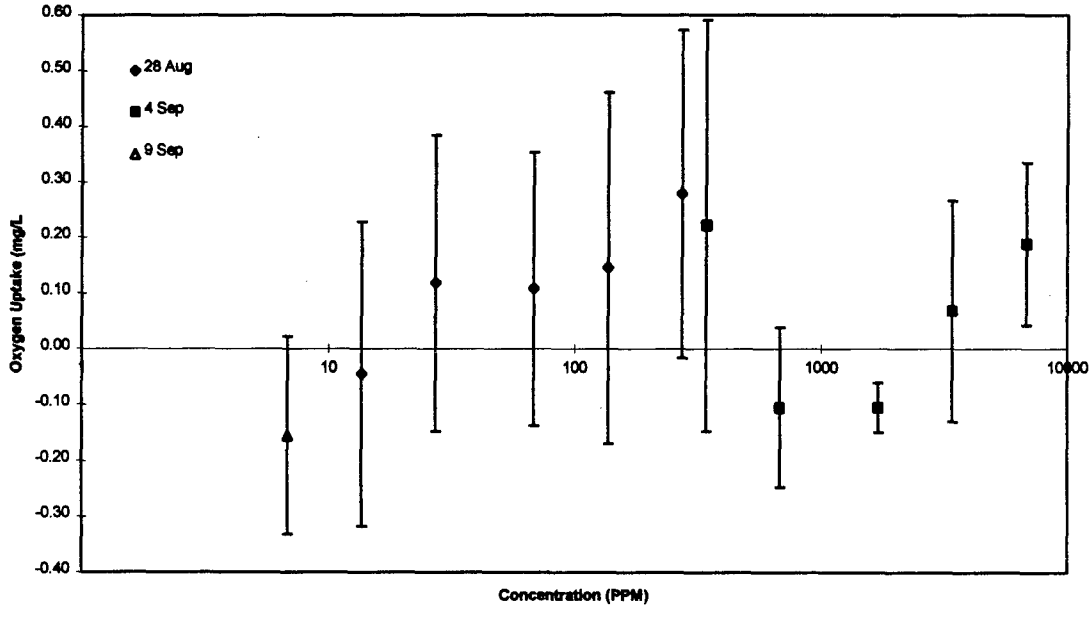
Dipropylene Glycol Tests

The results of the Dipropylene Glycol five-day BOD tests are presented in a tabular form in Table 13 and plotted in Figure 3.

TABLE 13
BOD5 DIPROPYLENE GLYCOL RESULTS

Test Date	Conc. (ppm)	O_2 Consumed (mg/L)	BOD (g/g)	BOD/ThOD (%)
08/26/96	14	-0.05 ± 0.27	< 0.14	< 7.5
	27	0.12 ± 0.27	< 0.07	< 3.9
	68	0.06 ± 0.42	< 0.03	< 1.5
	136	0.15 ± 0.32	< 0.015	< 0.8
	273	0.28 ± 0.29	< 0.007	< 0.4
09/04/96	341	0.22 ± 0.37	< 0.006	< 0.3
	682	-0.11 ± 0.14	< 0.003	< 0.15
	1704	-0.11 ± 0.04	< 0.0012	< 0.06
	3408	0.07 ± 0.20	< 0.0006	< 0.03
	6816	0.19 ± 0.15	< 0.0003	< 0.02
09/09/96	7	-0.16 ± 0.18	< 0.28	< 1.5

FIGURE 3
5-DAY OXYGEN UPTAKES FOR DPG



No significant evidence of biodegradability was observed for dipropylene glycol during the five day period over a broad range of concentrations. This seems to conflict with Bridie's result of a BOD/ThOD of 5%. This difference may be attributable to

differences in microbial populations. Bridie used the effluent of a 'biological sanitary treatment plant' as biological seed (Bridie et al., 1979B).

Glycerol Formal Testing

The results of the glycerol formal tests are presented in Table 14. BOD_5 values are given in milligrams of oxygen consumed per liter of glycerol formal. The results from 18 August indicate that a reportable biochemical oxygen demand was exerted (Table 14). The combination of relatively constant BOD values (Figure 4) and oxygen uptake values (Figure 5) that steadily increase with concentration indicate that glycerol formal acts much like a standard, non-limited, non-toxic chemical at the tested concentrations. However, when the extremely low (less than 0.5%) BOD_5/ThOD values are coupled with the knowledge that the glycerol formal sold from Aldrich was only 98.8% pure, it was entirely plausible that the oxygen demand exerted was the result of impurities.

TABLE 14
BOD_5 GLYCEROL FORMAL RESULTS

Test Date	Conc. (ppm)	O_2 Consumed (mg/L)	BOD_5 (mg/L)	BOD/ThOD (%)
08/11/96	4	0.11 ± 0.16	< 600,000	< 52.4
	8	0.12 ± 0.19	< 300,000	< 26.2
	20	0.32 ± 0.29	< 120,000	< 10.5
	40	0.26 ± 0.09	< 60,000	< 5.2
	80	0.32 ± 0.08	< 30,000	< 2.6
	201	0.72 ± 0.22	4,340 ± 1,310**	0.31 ± 0.09**
08/18/96	201	0.65 ± 0.03	3,900 ± 190**	0.27 ± 0.01**
	602	2.21 ± 0.21	4,410 ± 420	0.32 ± 0.03
	1003	4.01 ± 0.56	3,760 ± 630	0.26 ± 0.04
09/09/96	8	-0.13 ± 0.09	< 300,000	< 2.6

** Reading is technically too small (less than 2 mg/l depleted).

FIGURE 4
5-DAY BOD VALUES FOR GLYCEROL FORMAL

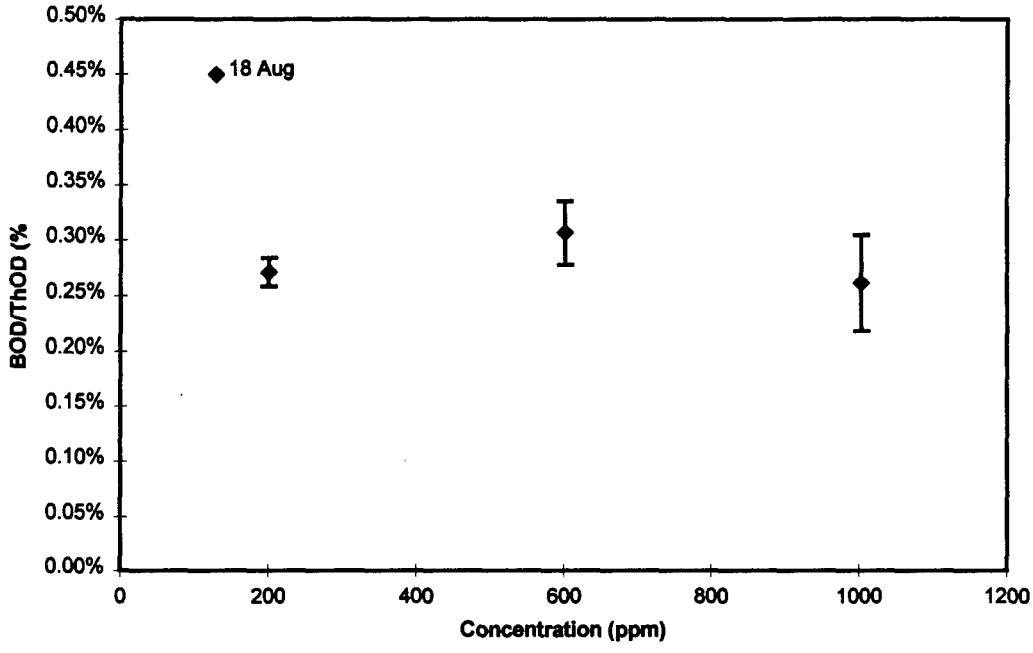
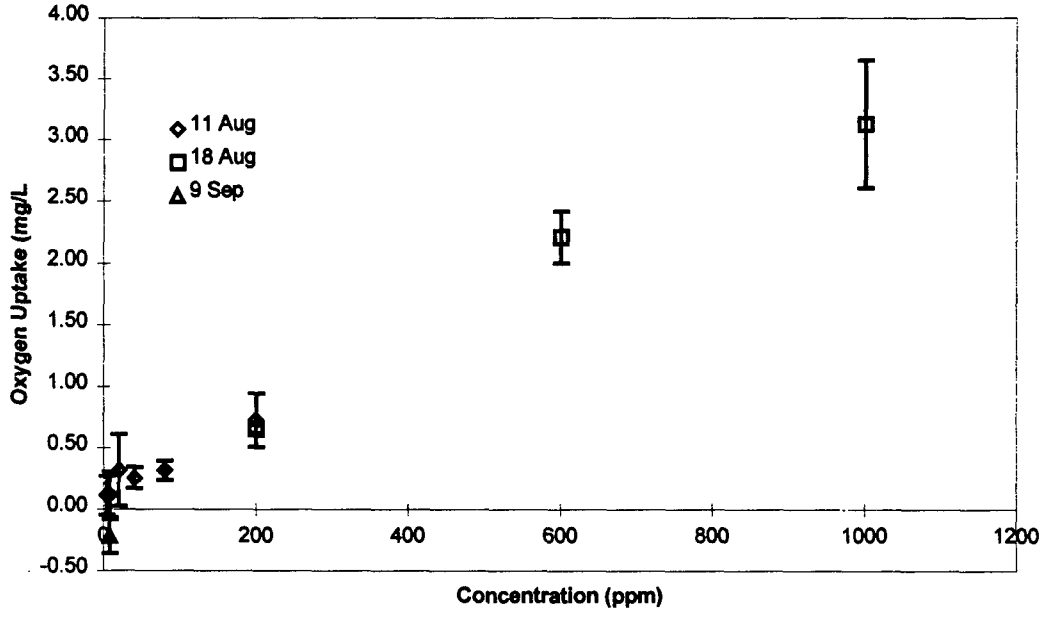


FIGURE 5
OXYGEN UPTAKE FOR GLYCEROL FORMAL



Acclimated Seed Tests

The results of the acclimated seed tests are presented in Table 15.

TABLE 15
ACCLIMATED SEED BOD_5 RESULTS

Test Date	Chemical	Conc. (ppm)	O_2 Consumed (mg/L)	BOD/ThOD (%)
9/11/96	DiEGME	7	0.48 ± 0.22	< 17
	DiEGME	14	0.84 ± 0.27	< 8.3
9/11/96	DPG	7	0.26 ± 0.17	< 15
	DPG	14	0.34 ± 0.17	< 7.5
09/20/96	GF	40	0.04 ± 0.37	< 5.2
	GF	80	0.11 ± 0.40	< 2.6

Although the glucose/glutamic acid check was acceptable for all of these tests, less oxygen consumption was seen for DiEGME samples than was seen in the standard tests using unacclimated seed. It is not clear whether or not the DPG or GF tests showed different oxygen uptakes as they reading were below the acceptable range. Possible explanations include that either the seed was not very healthy or acclimation was somewhat difficult. It is likely that since these chemicals are not very volatile, they or their metabolites had accumulated in the biological seed solution at inhibitory concentrations. The raw data for the GF test shows that less DO was consumed in the seed controls that had higher concentrations of seed (Appendix B). This could be interpreted as self inhibition.

Inspection of the acclimated seed cultures in the 1000 ml Erlenmeyer flasks revealed noticeable qualitative differences. The DiEGME culture was the most turbid (a sign of strong microbial growth). The DPG culture appeared to have significant growth on the walls of flask but less turbidity in the liquid culture than DiEGME. This is should

be noted. If the microorganism which degrade DPG tends to be fixed to surfaces, the use of a liquid biological seed may provide weak results. The glycerol formal culture had the least activity of all and appeared to be extremely clear.

Inhibition Tests

The inhibition tests did not show any significant decrease in oxygen demand when any of the FSIIs were added to samples containing glucose/glutamic acid and biological seed (Table 16). These DiEGME results were calculated by subtracting the oxygen demand for DiEGME from the oxygen demand of both DiEGME and glucose/glutamic acid. The variation in this sample is due to the inherent variability in DiEGME degradation. The oxygen demand of dipropylene glycol and glycerol formal were considered to be zero and the BOD of glucose/glutamic acid was calculated as if it were the sole substrate (Appendix C). The lack evidence of inhibition is a significant finding as it provides additional evidence that these chemicals would not interfere with the operation of a biological treatment plant at similar concentrations.

TABLE 16
INHIBITION TEST RESULTS

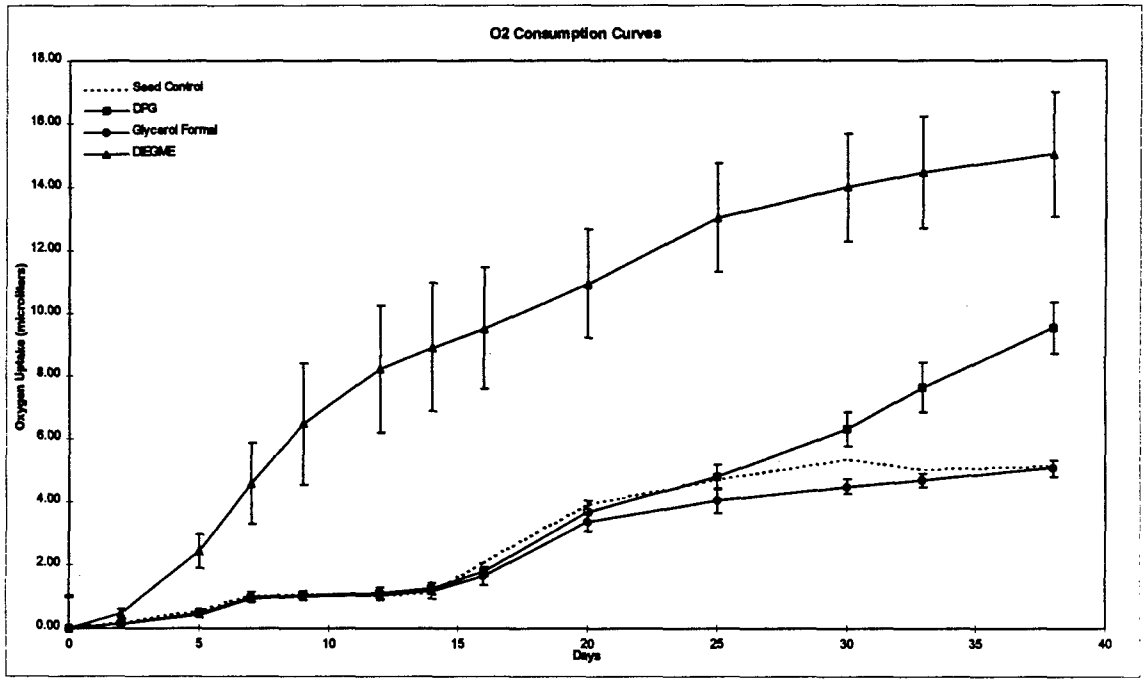
Test Chemical	Test Chemical Concentration (ppm)	Glucose/Glutamic Acid Solution BOD (mg/L)
G/G Control	-	177 ± 1
DiEGME +G/G	7	236 ± 87
Dipropylene Glycol + G/G	7	179 ± 7
Glycerol Formal + G/G	7	189 ± 10

It is important to realize that these tests only provide a preliminary, qualitative screening of the microbial toxicity of these chemicals and that extrapolation to a full scale sewage treatment plant is problematic (Pajak, 1977).

Long-term BOD Tests

The results of the first long-term BOD test are presented in Figure 6 in the form of oxygen consumption curves.

FIGURE 6
LONG-TERM OXYGEN CONSUMPTION CURVES

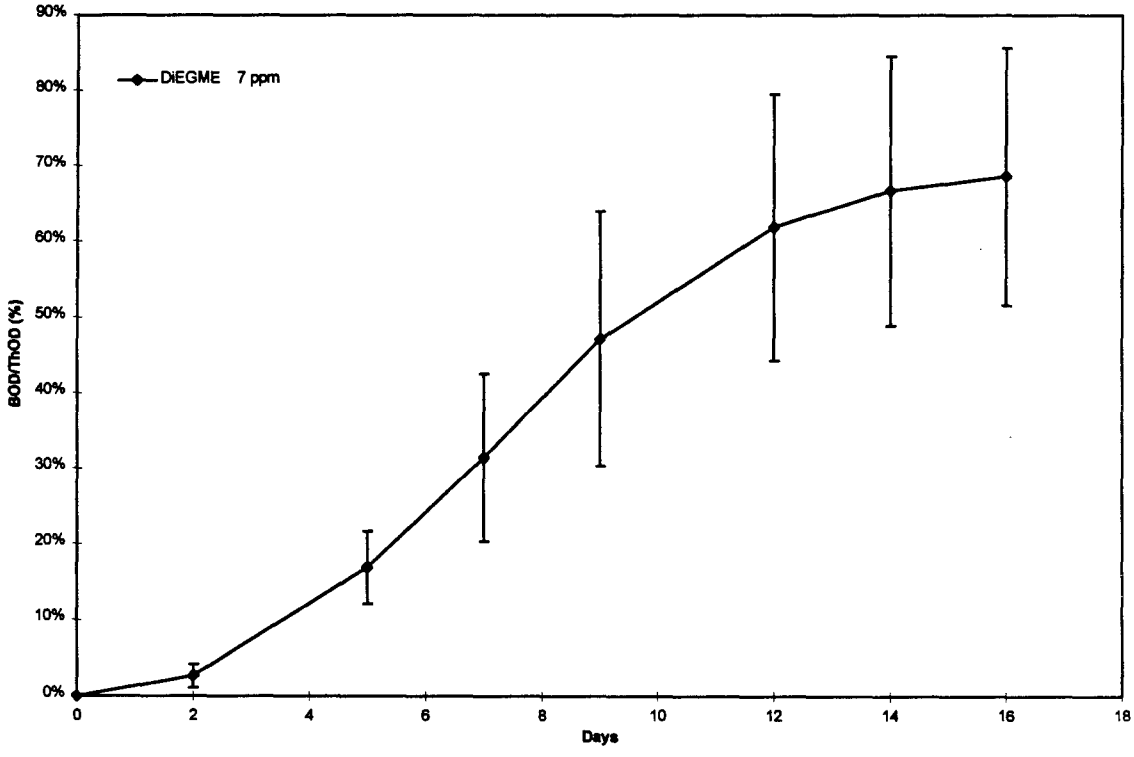


The results of the first test confirm that DiEGME appears to be relatively degradable. Glycerol formal did not show any noticeable increase in oxygen uptake with respect to the control. Dipropylene glycol did not show any increase in oxygen demand over the control until after the twenty-fifth day of testing. This appears to be an

remarkably long lag-time. The mechanisms responsible for such a lag time are not obvious and further research into this may be warranted.

A plot of the biodegradation dynamics of the DiEGME sample data is presented in Figure 7. DiEGME appears to degrade fairly quickly but not completely. The shape of the oxygen consumption curve follows the theoretical pattern of an exponential phase as microbial populations expand, followed by a linear region in which a constant microbial population and a constant substrate removal rate are present, followed by a leveling off as the substrate is depleted. As only one of the control samples was trusted (see page 28), a judgment was made to use the DPG and GF samples as pseudo-controls in the construction of this figure. This practice was probably safe for the first 14 days but slightly less conservative for later measurements.

FIGURE 7
DIEGME BIODEGRADATION CURVE



Second Long-Term BOD Test. After the first test failed to show activity for the other two FSII in the first twenty days, a second test was conducted for DiEGME at three different concentrations. The results are presented in an oxygen uptake format in Figure 8 and in BOD values in Figure 9. As reliable replicates of the control were used, the oxygen uptake results presented do have the contribution of the biological seed subtracted out.

TABLE 17
LONG TERM BOD_X VALUES

Date	Compound	Conc. (ppm)	BOD_5/ThOD (%)	BOD_{10}/ThOD (%)	BOD_{15}/ThOD (%)	BOD_{20}/ThOD (%)
09/04/96	GF	7.0	- 0 -	- 0 -	- 0 -	- 0 -
09/04/96	DPG	7.0	- 0 -	- 0 -	- 0 -	- 0 -
09/04/96	DiEGME	7.0	17 ± 5	47 ± 7	67 ± 18	67 ± 18
10/01/96	DiEGME	3.5	30 ± 8*	53 ± 9	67 ± 11	87 ± 11
10/01/96	DiEGME	7.0	17 ± 2	34 ± 3	52 ± 5	66 ± 4
10/01/96	DiEGME	14	13 ± 3	41 ± 5**	62 ± 6	67 ± 5

* Less than 2 mg/L depleted.
** Three samples had less than 1 mg/L remaining, these were not used in the remaining tests.

If one compares the DiEGME data for the first five days to the data for days five to ten, a few things can be learned. For the samples with concentrations of 4 ppm, less oxygen was consumed in the second five days. For the 7 ppm samples roughly the same amount of oxygen was consumed. For 14 ppm, more oxygen demand was exerted for the second five days. This supports the assertion that population growth or enzyme inducement was necessary at greater concentrations. For 4 ppm more than enough microbes were available. For 14 ppm, growth or enzyme production was necessary to fully utilize the substrate.

FIGURE 8
DIEGME OXYGEN UPTAKE

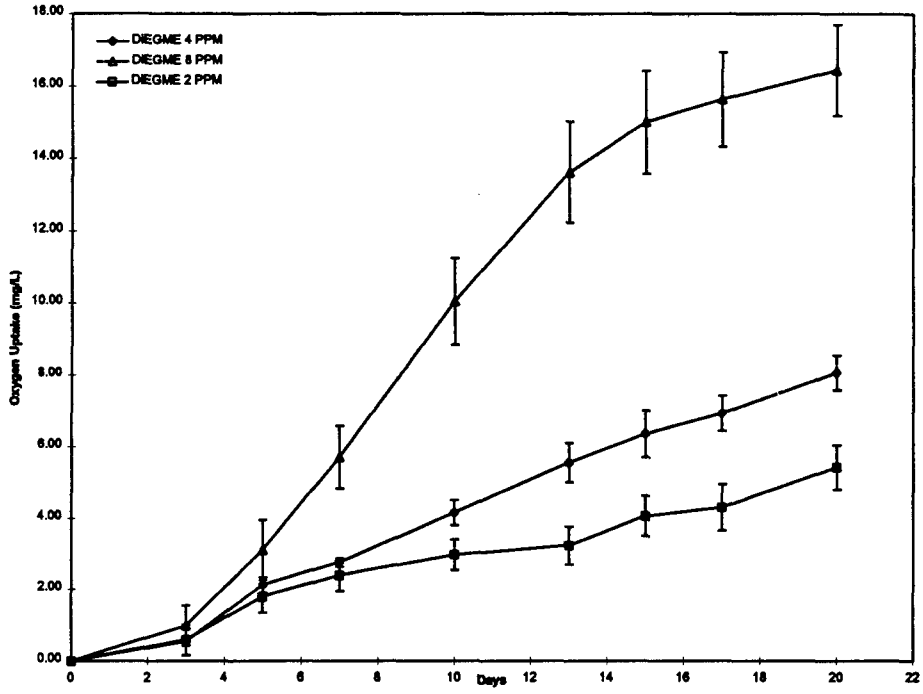
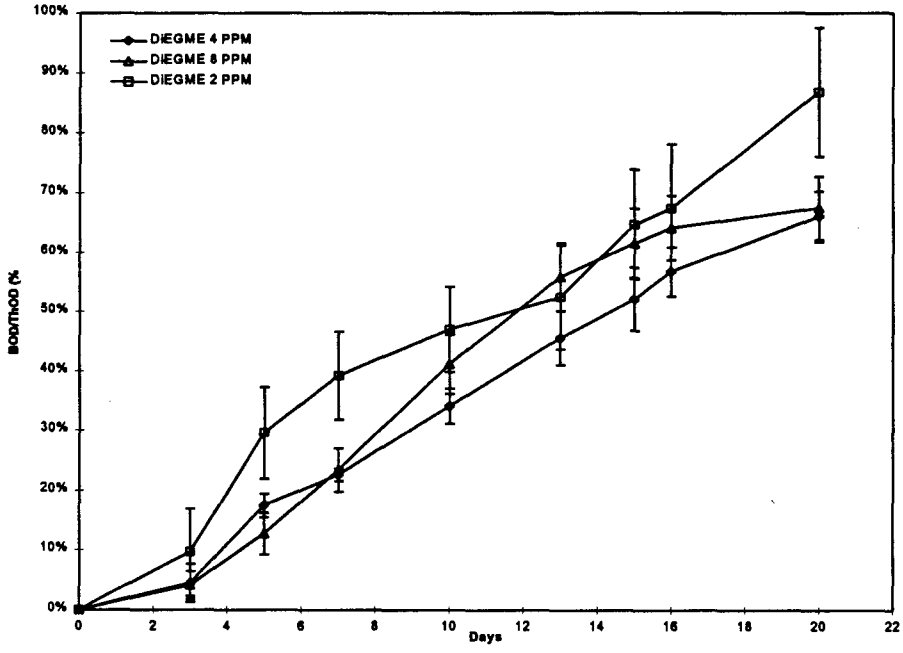


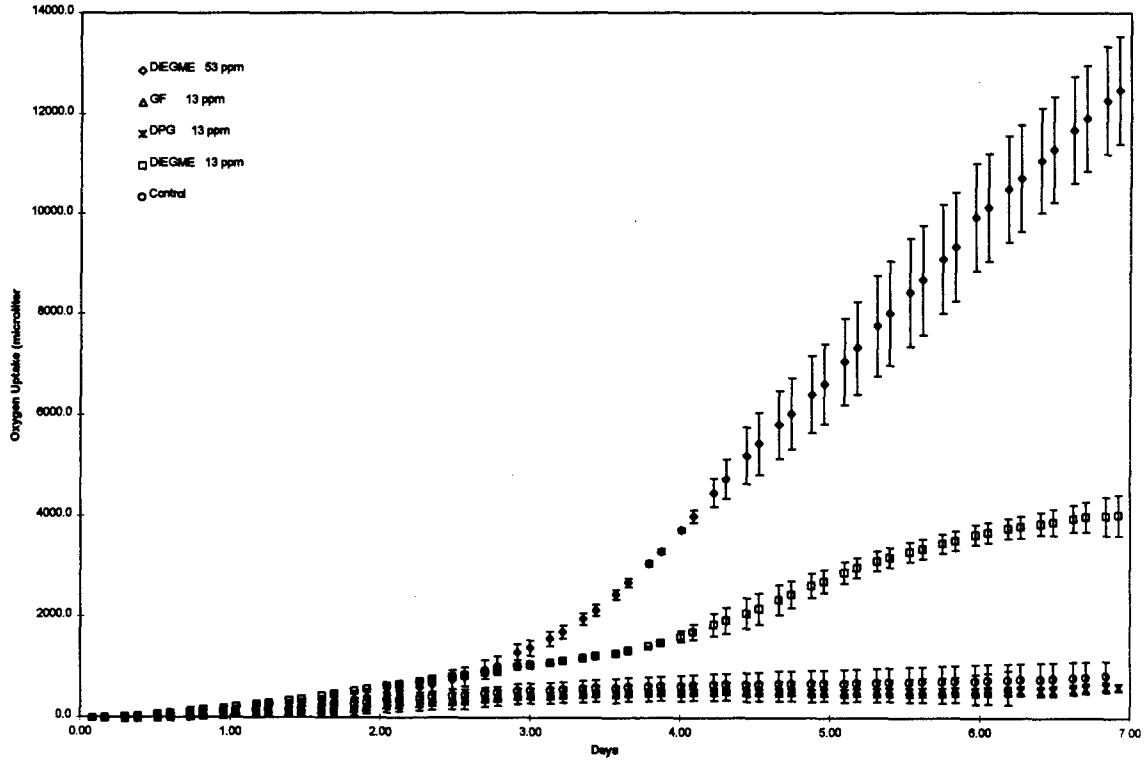
FIGURE 9
DIEGME LONG-TERM BOD VALUES



Respirometer Testing

The results of the automatic respirometer tests are provided in Figure 10. The respirometer test appeared to work successfully until the sixth day. Erratic readings occurred on two channels on this day and spread to the remaining channels in the following days for unknown reasons. Potential causes of these faulty readings may have been excess moisture in the system and/or a drop in the room temperature where the test equipment was housed. This drop in temperature may have caused a leak in some of the fittings. The data prior to the sixth day appears to be reliable.

FIGURE 10
AUTOMATIC RESPIROMETRY FSII PLOT



The DiEGME samples behaved in much the same way as they did in the BOD testing. The two concentrations depleted similar amounts of oxygen until a certain lag time was reached and after that point the rate of consumption increased dramatically. This lag time was significantly decreased in the respirometer testing because the temperature in this experiments was set at 29°C ± 1°C as opposed to the 20°C ± 1°C mandated for the BOD tests. The glycerol formal and dipropylene glycol samples showed no evidence of degradation in the first six days, the valid portion of this test.

Agar Diffusion Toxicity Test

A photograph of the results of the Petri dish toxicity test and an identification key are provided in Appendix D. The photograph was taken after approximately thirty hours of incubation at 29° C.

DiEGME. The DiEGME dishes had relatively large circles in which no growth occurred. This inner circle was completely bare which would indicate microbial toxicity at high concentrations. There appeared to be denser growth outside of the initial bare circle which would indicate that the microorganisms may have utilized the DiEGME at lower concentrations.

Dipropylene Glycol. The dipropylene glycol plates showed a noticeable circle in which growth was very thin, but not completely bare as it was for DiEGME which would indicate moderate microbial toxicity. An increase in growth had not occurred outside the bare regions as it did with DiEGME, which would support the BOD_5 results of no biodegradation occurring.

Glycerol Formal Plates. The glycerol formal plates showed very little inhibition of growth, although a small circle may be seen immediately around the center hole. This small circle may indicate some toxicity or merely a displacement of microbes by the glycerol formal droplet. The lack of any additional growth would be consistent with the BOD_5 findings of a lack of biodegradability.

Control Plates. The controls had microbial growth spread evenly across the surface of the plates.

Agar Diffusion Toxicity Test Summary. These results appear to indicate that DiEGME did inhibit the growth of microbes at high concentrations but appeared to be utilized as food at lower concentrations. Dipropylene glycol appeared to either slow the growth of all microorganisms or inhibit the growth of select microorganisms at higher concentrations. Glycerol Formal showed very little inhibitory effect on the sewage microorganisms.

V. Conclusions and Recommendations

The results of the six different experiments provide a much more complete picture of the biodegradation characteristics of FSIIs than could be accomplished through any single test. As these tests where conducted in near identical conditions for each substance, a comparative evaluation of these FSIIs is made much more valid.

DiEGME Biodegradation Characteristics

DiEGME appeared to be moderately but not completely biodegradable. The results would lead to a reportable BOD_5/ThOD ratio of about 9%, but this is relatively conservative. Concentrations below the reportable range of the test appear to exert a higher oxygen demand and the oxygen demand also appears to increase substantially after the end of the test period of five days.

DiEGME appears to be fairly toxic to microorganisms at high concentrations. This appears to confirm the current practice of relying on DiEGME to act as a biocide in tank bottoms. It also appears to not be very inhibitory to microbial activity at low concentrations which should help to alleviate fears that it might interfere with the operation of POTWs.

Glycerol Formal Biodegradation Characteristics

Glycerol Formal did not show any evidence of biodegradation and appeared to be the least microbially toxic compound. The agar diffusion toxicity tests show minimal or no inhibition at high concentrations and the inhibitory BOD_5 tests did not show any

interference. This provides some evidence that it may behave in a similar fashion to the structurally similar dioxane. All in all, there was no evidence that glycerol formal could be removed from a treatment plant, but no evidence was provided which would indicate that it would adversely impact treatment plant operations.

Dipropylene Glycol Biodegradation Characteristics

The only evidence of biodegradation of dipropylene glycol came after twenty five days. The consumption of oxygen after these twenty five days was relatively rapid. This would seem to indicate that the bacteria responsible for the degradation were not present in the biological seed in concentrations that were high enough to effectively biodegrade DPG. However, it does raise the possibility that if such a microbe might thrive in a wastewater treatment plant's activated sludge, then some removal of dipropylene glycol might be achieved.

The high concentration toxicity test showed that dipropylene glycol appeared to be toxic, but not to the same degree as DiEGME. The low concentration inhibition test did not present evidence that dipropylene glycol would interfere with biological processes.

Disposal

The disposal of a few gallons of FSII-saturated tank bottom wastes to a relatively small WWTP can have a significant impact. Five gallons of DiEGME dumped to a medium sized installation WWTP (0.7 MGD with an aeration tank retention time of 6 hours), will produce an aeration tank concentration of about 30 ppm. This can create a

potential problem by loading the biological treatment processes substantially (raising the ThOD of the tank by about 50 mg/L) and could conceivably cause the effluent BOD levels to rise. If the other two FSIIs, dipropylene glycol and glycerol formal, prove to not be treatable, a similar scenario might result in either chemical passing through the plant untreated. This would raise the effluent COD similarly, possibly contaminate drinking water supplies or sensitive aquatic environments. Whether or not these wastes are acceptable for disposal from strictly a BOD/COD viewpoint is a question which should be addressed on a case by case basis. Variables to consider include the size of the receiving treatment plant, its retention time, and all of the variables that impact the volume of tank bottoms produced; including transportation of jet fuel to the base, variations in the flying mission, and variations in weather conditions.

In situations where the ability of biological treatment to successfully treat FSIIs is in doubt, other treatment or disposal routes should be considered. Given the remarkably high concentrations of FSII found in tank bottoms, treatment or disposal instead of dilution to a wastewater treatment plant might be the best approach.

Recommendations

In conclusion, the disposal of these wastes would require analysis of the volume of wastes and the size of the receiving treatment facility. Little evidence of toxicity to a treatment plant was found. DiEGME would probably be largely removed in a treatment plant. However, the removal of DPG and, to a greater extent, glycerol formal is very doubtful. As DiEGME and DPG have proved to be resilient enough in the environment

to contaminate drinking water supplies (Howard et al., 1989), it would be prudent to investigate other methods of disposal than sending to a POTW.

Future Research

During the acclimated seed tests it was noted that a great deal of microbial growth occurred on the surfaces of the walls of the flasks. A BOD test could be developed in which microorganisms are fixed to a surface as opposed using liquid biological seed. Large glass beads or filter packing material could be placed in the flasks where the microorganisms are acclimated to the test compound with the intention of growing a biofilm on the surfaces of the beads or filter packing. These could then be placed in the test bottles and treated the same as liquid seed.

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	8/18/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Std Deviation	Corrected O2 Depletion	Correct O2 Std Dev	Raw BOD (mg/L)	Raw BOD Std Deviation	**Actual BOD (mg/L)**	**Actual BOD Std Deviation**
Dilution Water	0.00	0.0E+00	8.54	8.42	0.12							
Dilution Water	0.00	0.0E+00	8.51	8.46	0.05							
					0.08							
Seed Control	3.00	1.0E-02	8.43	7.70	0.73				73			
Seed Control	6.00	2.0E-02	8.35	6.59	1.76				88			
Seed Control	9.00	3.0E-02	8.32	5.49	2.83				94			
				Seed DO	0.85	0.11			85	11		
Glucose-Glutamic Acid	6.00	2.0E-02	8.41	3.58	4.83		3.98		199			
Glucose-Glutamic Acid	6.00	2.0E-02	8.42	3.56	4.86		4.01		200			
DiEGME	0.004	1.3E-05	8.25	3.40	4.85		4.00	0.11	363,750		299,917	8,217
DiEGME	0.004	1.3E-05	8.30	3.19	5.11		4.26	0.11	383,250		319,417	8,217
DiEGME	0.004	1.3E-05	8.26	4.14	4.12		3.27	0.11	309,000		245,167	8,217
		1.3E-05			4.69	0.51	3.84	0.52	352,000	38,494	**288,167**	**39,362**
DiEGME	0.008	2.7E-05	8.30	3.60	4.70		3.85	0.11	176,250		144,333	4,109
DiEGME	0.008	2.7E-05	8.30	3.51	4.79		3.94	0.11	179,625		147,708	4,109
DiEGME	0.008	2.7E-05	8.28	5.29	2.99		2.14	0.11	112,125		80,208	4,109
		2.7E-05			4.16	1.01	3.31	1.02	156,000	38,034	**124,083**	**38,258**
DiEGME	0.012	4.0E-05	8.27	4.10	4.17		3.32	0.11	104,250		82,972	2,739
DiEGME	0.012	4.0E-05	8.26	4.06	4.20		3.35	0.11	105,000		83,722	2,739
DiEGME	0.012	4.0E-05	8.25	4.52	3.73		2.88	0.11	93,250		71,972	2,739
		4.0E-05			4.03	0.26	3.18	0.29	100,833	6,578	**79,556**	**7,126**

Completion Date:	7/31/96											
Dilution Water	0.00	0.0E+00	8.18	7.89	0.29							
Dilution Water	0.00	0.0E+00	8.18	7.93	0.25							
					0.27							
Seed Control	3.00	1.0E-02	8.08	6.98	1.10				110			
Seed Control	6.00	2.0E-02	8.00	6.03	1.97				99			
Seed Control	9.00	3.0E-02	8.00	5.19	2.81				94			
				Seed DO	1.01	0.08			101	8		
Glucose-Glutamic Acid	6.00	2.0E-02	8.06	2.93	5.13		4.28		214			
Glucose-Glutamic Acid	6.00	2.0E-02	8.05	2.83	5.22		4.37		218			
DiEGME	0.005	1.7E-05	8.05	2.89	5.16		4.15	0.08	309,600		249,167	5,034
DiEGME	0.005	1.7E-05	8.03	3.43	4.60		3.59	0.08	276,000		215,567	5,034
DiEGME	0.005	1.7E-05	8.02	4.38	3.64		2.63	0.08	218,400		157,967	5,034
		1.7E-05			4.47	0.77	3.46	0.77	268,000	46,123	**207,567**	**46,397**
DiEGME	0.010	3.3E-05	8.00	3.60	4.40		3.39	0.08	132,000		101,783	2,517
DiEGME	0.010	3.3E-05	8.02	4.06	3.96		2.95	0.08	118,800		88,583	2,517
DiEGME	0.010	3.3E-05	8.02	3.86	4.16		3.15	0.08	124,800		94,583	2,517
		3.3E-05			4.17	0.22	3.17	0.24	125,200	6,609	**94,983**	**7,072**
DiEGME	0.025	8.3E-05	7.98	0.11	7.87		6.86	0.08	94,428		82,341	1,007
DiEGME	0.025	8.3E-05	7.96	0.10	7.86		6.85	0.08	94,320		82,233	1,007
DiEGME	0.025	8.3E-05	7.97	0.02	7.95		6.95	0.08	95,448		83,361	1,007
		8.3E-05			7.89	0.05	6.89	0.10	94,732	622	**82,645**	**1,184**
DiEGME	0.050	1.7E-04	7.93	0.14	7.79		6.78	0.08	46,740		40,697	503
DiEGME	0.100	3.3E-04	7.73	0.10	7.63		6.62	0.08	22,890		19,868	252

Completion Date:	9/9/96											
Dilution Water	0.00	0.0E+00	8.58	8.40	0.18							
Dilution Water	0.00	0.0E+00	8.59	8.42	0.17							
					0.18							
Seed Control	2.50	8.3E-03	8.53	7.97	0.56				67			
Seed Control	6.00	2.0E-02	8.44	7.25	1.19				60			
Seed Control	9.00	3.0E-02	8.37	6.15	2.22				74			

				Seed DO	0.56	0.06			67	7		
Glucose-Glutamic Acid	6.00	2.0E-02	8.30	4.10	4.20		3.64		182			
Glucose-Glutamic Acid	6.00	2.0E-02	8.31	4.27	4.04		3.48		174			
DiEGME	0.002	6.7E-06	8.24	5.38	2.86		2.30	0.06	429,000		345,375	9,068
DiEGME	0.002	6.7E-06	8.25	6.05	2.20		1.64	0.06	330,000		246,375	9,068
DiEGME	0.002	6.7E-06	8.26	6.50	1.76		1.20	0.06	264,000		180,375	9,068
DiEGME	0.002	6.7E-06	8.27	5.39	2.88		2.32	0.06	432,000		348,375	9,068
		6.7E-06			2.43	0.54	1.87	0.55	363,750	81,659	**280,125**	**82,161**
Completion Date:	10/6/96											
Dilution Water	0.00	0.0E+00	8.02	7.91	0.11							
Dilution Water	0.00	0.0E+00	8.03	7.83	0.20							
					0.15							
Seed Control	2.50	8.3E-03	7.96	6.98	0.98				118			
Seed Control	2.50	8.3E-03	7.95	6.86	1.09				131			
Seed Control	2.50	8.3E-03	7.91	7.02	0.89				107			
				Seed DO	0.99	0.10			118	12		
Glucose-Glutamic Acid	6.00	2.0E-02	7.89	3.38	4.51		3.52		176			
Glucose-Glutamic Acid	6.00	2.0E-02	7.83	3.30	4.53		3.54		177			
Glucose-Glutamic Acid	6.00	2.0E-02	7.87	3.32	4.55		3.56		178			
DiEGME	0.002	6.7E-06	7.80	4.55	3.25		2.26	0.06	487,500		339,500	9,068
DiEGME	0.002	6.7E-06	7.80	5.00	2.80		1.81	0.06	420,000		272,000	9,068
DiEGME	0.002	6.7E-06	7.78	4.94	2.84		1.85	0.06	426,000		278,000	9,068
DiEGME	0.002	6.7E-06	7.78	5.02	2.76		1.77	0.06	414,000		266,000	9,068
DiEGME	0.002	6.7E-06	7.82	4.80	3.02		2.03	0.06	453,000		305,000	9,068
DiEGME	0.002	6.7E-06	7.80	4.99	2.81		1.82	0.06	421,500		273,500	9,068
		6.7E-06			2.91	0.19	1.93	0.21	437,000	28,233	**289,000**	**31,982**
DiEGME	0.004	1.3E-05	7.80	3.67	4.13		3.14	0.06	309,750		235,750	4,534
DiEGME	0.004	1.3E-05	7.84	3.77	4.07		3.08	0.06	305,250		231,250	4,534
DiEGME	0.004	1.3E-05	7.85	3.92	3.93		2.94	0.06	294,750		220,750	4,534
DiEGME	0.004	1.3E-05	7.85	3.98	3.87		2.88	0.06	290,250		216,250	4,534
DiEGME	0.004	1.3E-05	7.85	5.46	2.39		1.40	0.06	179,250		105,250	4,534
DiEGME	0.004	1.3E-05	7.84	2.87	4.97		3.98	0.06	372,750		298,750	4,534
		1.3E-05			3.89	0.84	2.91	0.84	292,000	62,824	**218,000**	**63,272**
DiEGME	0.001	3.3E-06	7.83	5.28	2.55		1.56	0.06	765,000		469,000	18,137
DiEGME	0.001	3.3E-06	7.86	4.98	2.88		1.89	0.06	864,000		568,000	18,137
DiEGME	0.001	3.3E-06	7.86	5.08	2.78		1.79	0.06	834,000		538,000	18,137
DiEGME	0.001	3.3E-06	7.84	6.07	1.77		0.78	0.06	531,000		235,000	18,137
DiEGME	0.001	3.3E-06	7.81	4.78	3.03		2.04	0.06	909,000		613,000	18,137
DiEGME	0.001	3.3E-06	7.83	5.29	2.54		1.55	0.06	762,000		466,000	18,137
		3.3E-06			2.59	0.45	1.61	0.46	777,500	133,511	**481,500**	**136,851**
Completion Date:	7/23/96											
Dilution Water	0.00	0.0E+00	8.01	7.97	0.04							
Dilution Water	0.00	0.0E+00	8.02	7.85	0.17							
					0.11							
Seed Control	1.30	4.3E-03	7.97	7.63	0.34				78			
Seed Control	5.80	1.9E-02	7.85	5.75	2.10				109			
Seed Control	8.80	2.9E-02	7.73	5.59	2.14				73			
				Seed DO	0.38	0.08			87	19		
Glucose-Glutamic Acid	6.00	2.0E-02	7.89	7.93	3.30		2.31		116			
Glucose-Glutamic Acid	6.00	2.0E-02	7.83	7.96	3.89		2.90		145			
DiEGME	0.005	1.7E-05	7.92	5.65	2.27		1.26	0.08	136,200		75,767	5 034
DiEGME	0.005	1.7E-05	7.92	6.92	1.00		-0.01	0.08	60,000		-433	5 034
DiEGME	0.005	1.7E-05	7.89	6.33	1.56		0.55	0.08	93,600		33,167	5 034
		1.7E-05			1.61	0.64	1.23	0.64	96,600	38,188	**74,063**	**38,513**
DiEGME	0.010	3.3E-05	7.88	3.96	3.92		3.54	0.08	117,600		106,332	2 496
DiEGME	0.010	3.3E-05	7.89	6.06	1.83		1.45	0.08	54,900		43,632	2 496
DiEGME	0.010	3.3E-05	7.91	6.82	1.09		0.71	0.08	32,700		21,432	2 496
		3.3E-05			2.28	1.47	1.90	1.47	68,400	44,031	**57,132**	**44,101**
DiEGME	0.025	8.3E-05	7.88	0.10	7.78		7.40	0.08	93,360		88,853	998
DiEGME	0.025	8.3E-05	7.88	0.06	7.82		7.44	0.08	93,840		89,333	998
DiEGME	0.025	8.3E-05	7.87	0.07	7.80		7.42	0.08	93,600		89,093	998
		8.3E-05			7.80	0.02	7.42	0.09	93,600	240	**89,093**	**1,027**
DiEGME	0.050	1.7E-04	7.93	0.14	7.79		7.41	0.08	46,740		44,486	499
DiEGME	0.100	3.3E-04	7.73	0.10	7.63		7.25	0.08	22,890		21,763	250

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	8/11/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Std Deviation	Corrected O2 Depletion	Correct O2 Std Dev	Raw BOD (mg/L)	Raw BOD Std Deviation	Actual BOD (mg/L)	Actual BOD Std Deviation
Dilution Water	0.00	0.0E+00	8.10	7.92	0.18							
Dilution Water	0.00	0.0E+00	8.10	7.92	0.18							
					0.18							
Seed Control	2.00	6.7E-03	8.03	7.34	0.69				104			
Seed Control	6.00	2.0E-02	7.94	5.95	1.99				100			
Seed Control	9.00	3.0E-02	7.80	4.81	2.99				100			
				Seed DO	0.67	0.02			101	2		
Glucose-Glutamic Acid	6.00	2.0E-02	7.93	3.69	4.24		3.57		178			
Glucose-Glutamic Acid	6.00	2.0E-02	7.98	3.69	4.29		3.62		181			
Glycerol Formal	0.001	3.3E-06	8.00	7.17	0.83		0.16	0.02	249,000		47,222	4,526
Glycerol Formal	0.001	3.3E-06	7.96	7.35	0.61		-0.06	0.02	183,000		-18,778	4,526
Glycerol Formal	0.001	3.3E-06	7.96	7.04	0.92		0.25	0.02	276,000		74,222	4,526
		3.3E-06			0.79	0.16	0.11	0.16	236,000	47,843	**34,222**	**48,057**
Glycerol Formal	0.002	6.7E-06	7.92	6.91	1.01		0.34	0.02	151,500		50,611	2,263
Glycerol Formal	0.002	6.7E-06	7.90	7.20	0.70		0.03	0.02	105,000		4,111	2,263
Glycerol Formal	0.002	6.7E-06	7.96	7.29	0.67		0.00	0.02	100,500		-389	2,263
		6.7E-06			0.79	0.19	0.12	0.19	119,000	28,236	**18,111**	**28,326**
Glycerol Formal	0.005	1.7E-05	7.95	6.55	1.40		0.73	0.02	84,000		43,644	905
Glycerol Formal	0.005	1.7E-05	7.93	7.00	0.93		0.26	0.02	55,800		15,444	905
Glycerol Formal	0.005	1.7E-05	7.97	7.26	0.71		0.04	0.02	42,600		2,244	905
Glycerol Formal	0.005	1.7E-05	7.93	7.01	0.92		0.25	0.02	55,200		14,844	905
		1.7E-05			0.99	0.29	0.32	0.29	59,400	17,493	**19,044**	**17,516**
Glycerol Formal	0.010	3.3E-05	7.85	7.01	0.84		0.17	0.02	25,200		5,022	453
Glycerol Formal	0.010	3.3E-05	7.86	6.92	0.94		0.27	0.02	28,200		8,022	453
Glycerol Formal	0.010	3.3E-05	7.83	6.82	1.01		0.34	0.02	30,300		10,122	453
		3.3E-05			0.93	0.09	0.26	0.09	27,900	2,563	**7,722**	**2,603**
Glycerol Formal	0.020	6.7E-05	7.81	6.77	1.04		0.37	0.02	15,600		5,511	226
Glycerol Formal	0.020	6.7E-05	7.81	6.91	0.90		0.23	0.02	13,500		3,411	226
Glycerol Formal	0.020	6.7E-05	7.77	6.74	1.03		0.36	0.02	15,450		5,361	226
		6.7E-05			0.99	0.08	0.32	0.08	14,850	1,172	**4,761**	**1,193**
Glycerol Formal	0.050	1.7E-04	7.74	6.52	1.22		0.55	0.02	7,320		3,284	91
Glycerol Formal	0.050	1.7E-04	7.74	6.10	1.64		0.97	0.02	9,840		5,804	91
Glycerol Formal	0.050	1.7E-04	7.71	6.38	1.33		0.66	0.02	7,980		3,944	91
		1.7E-04			1.40	0.22	0.72	0.22	8,380	1,307	**4,344**	**1,310**

Completion Date:	8/18/96											
Dilution Water	0.00	0.0E+00	8.54	8.42	0.12							
Dilution Water	0.00	0.0E+00	8.51	8.46	0.05							
					0.08							
Seed Control	2.50	8.3E-03	8.43	7.70	0.73				88			
Seed Control	6.00	2.0E-02	8.35	6.59	1.76				88			
Seed Control	9.00	3.0E-02	8.32	5.49	2.83				94			
				Seed DO	0.75	0.03			90	4		
Glucose-Glutamic Acid	6.00	2.0E-02	8.41	3.58	4.83		4.16		204			
Glucose-Glutamic Acid	6.00	2.0E-02	8.42	3.56	4.86		4.19		206			
Glycerol Formal	0.050	1.7E-04	8.38	6.98	1.40		0.65	0.03	8,400		3,901	189
Glycerol Formal	0.050	1.7E-04	8.37	6.97	1.40		0.65	0.03	8,400		3,901	189
Glycerol Formal	0.050	1.7E-04	8.00	-	-		-	-			-	-
		1.7E-04			1.40	0.00	0.65	0.03	8,400	0	**3,901**	**189**
Glycerol Formal	0.150	5.0E-04	8.36	5.59	2.77		2.02	0.03	5,540		4,040	63
Glycerol Formal	0.150	5.0E-04	8.36	5.18	3.18		2.43	0.03	6,360		4,860	63
Glycerol Formal	0.150	5.0E-04	8.38	5.46	2.92		2.17	0.03	5,840		4,340	63
		5.0E-04			2.96	0.21	2.21	0.21	5,913	415	**4,414**	**420**
Glycerol Formal	0.250	8.3E-04	8.23	4.23	4.00		3.25	0.03	4,800		3,900	38
Glycerol Formal	0.250	8.3E-04	8.16	4.85	3.31		2.56	0.03	3,972		3,072	38
Glycerol Formal	0.250	8.3E-04	8.30	3.97	4.33		3.58	0.03	5,196		4,296	38

		8.3E-04			3.88	0.52	3.13	0.52	4,656	625	3,756	626
Completion Date:	9/9/96											
Dilution Water	0.00	0.0E+00	8.58	8.40	0.18							
Dilution Water	0.00	0.0E+00	8.59	8.42	0.17							
					0.18							
Seed Control	2.50	8.3E-03	8.53	7.97	0.56				67			
Seed Control	5.00	1.7E-02	8.44	7.25	1.19				71			
Seed Control	7.00	2.3E-02	8.37	6.15	2.22				95			
				Seed DO	0.65	0.13			78	15		
Glucose-Glutamic Acid	6.00	2.0E-02	8.30	4.10	4.20				178			
Glucose-Glutamic Acid	6.00	2.0E-02	8.31	4.27	4.04				170			
Glycerol Formal	0.002	6.7E-06	8.27	7.91	0.36		-0.29	0.13	54,000		-43,393	18,834
Glycerol Formal	0.002	6.7E-06	8.29	7.86	0.43		-0.22	0.13	64,500		-32,893	18,834
Glycerol Formal	0.002	6.7E-06	8.30	7.91	0.39		-0.26	0.13	58,500		-38,893	18,834
Glycerol Formal	0.002	6.7E-06	8.29	7.86	0.43		-0.22	0.13	64,500		-32,893	18,834
Glycerol Formal	0.002	6.7E-06	8.29	7.87	0.42		-0.23	0.13	63,000		-34,393	18,834
Glycerol Formal	0.002	6.7E-06	8.29	7.75	0.54		-0.11	0.13	81,000		-16,393	18,834
		6.7E-06			0.43	0.06	-0.22	0.14	64,250	9,169	**-33,143**	**20,948**

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	8/28/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Deplete Std Dev	Correct O2 Depletion	Correct O2 Std Dev	Raw BOD (mg/L)	Raw BOD Standard Deviation	**Actual BOD (mg/L)**	**Actual BOD Std Dev**
Dilution Water	0.00	0.0E+00	8.06									
Dilution Water	0.00	0.0E+00	8.06	7.96	0.10							
					0.10							
Seed Control	2.50	8.3E-03	7.93	6.70	1.23				148			
Seed Control	6.00	2.0E-02	7.88	5.84	2.04				102			
Seed Control	9.00	3.0E-02	7.74	4.84	2.90				97			
				Seed DO	0.96	0.23			115	28		
Glucose-Glutamic Acid	6.00	2.0E-02	7.85	3.18	4.67		3.71		185			
Glucose-Glutamic Acid	6.00	2.0E-02	7.80	3.14	4.66		3.70		185			
Dipropylene Glycol	0.004	1.3E-05	7.67	6.88	0.79		-0.17	0.23	59,250		-12,889	17,496
Dipropylene Glycol	0.004	1.3E-05	7.75	6.86	0.89		-0.07	0.23	66,750		-5,389	17,496
Dipropylene Glycol	0.004	1.3E-05	7.80	6.73	1.07		0.11	0.23	80,250		8,111	17,496
		1.3E-05			0.92	0.14	-0.05	0.27	68,750	10,642	**-3,389**	**20,479**
Dipropylene Glycol	0.008	2.7E-05	7.78	6.81	0.97		0.01	0.23	36,375		306	8,748
Dipropylene Glycol	0.008	2.7E-05	7.80	6.75	1.05		0.09	0.23	39,375		3,306	8,748
Dipropylene Glycol	0.008	2.7E-05	7.75	6.53	1.22		0.26	0.23	45,750		9,681	8,748
		2.7E-05			1.08	0.13	0.12	0.27	40,500	4,788	**4,431**	**9,973**
Dipropylene Glycol	0.020	6.7E-05	7.71	6.72	0.99		0.03	0.23	14,850		422	3,499
Dipropylene Glycol	0.020	6.7E-05	7.75	6.67	1.08		0.12	0.23	16,200		1,772	3,499
Dipropylene Glycol	0.020	6.7E-05	7.67	6.53	1.14		0.18	0.23	17,100		2,672	3,499
		6.7E-05			1.07	0.08	0.11	0.25	16,050	1,132	**1,622**	**3,678**
Dipropylene Glycol	0.040	1.3E-04	7.73	6.65	1.08		0.12	0.23	8,100		886	1,750
Dipropylene Glycol	0.040	1.3E-04	7.74	6.72	1.02		0.06	0.23	7,650		436	1,750
Dipropylene Glycol	0.040	1.3E-04	7.72	6.80	0.92		-0.04	0.23	6,900		-314	1,750
Dipropylene Glycol	0.040	1.3E-04	7.68	6.27	1.41		0.45	0.23	10,575		3,361	1,750
		1.3E-04			1.11	0.21	0.15	0.32	8,306	1,591	**1,092**	**2,365**
Dipropylene Glycol	0.080	2.7E-04	7.63	6.44	1.19		0.23	0.23	4,463		856	875
Dipropylene Glycol	0.080	2.7E-04	7.61	6.17	1.44		0.48	0.23	5,400		1,793	875
Dipropylene Glycol	0.080	2.7E-04	7.55	6.46	1.09		0.13	0.23	4,088		481	875
		2.7E-04			1.24	0.18	0.28	0.29	4,650	676	**1,043**	**1,106**

Completion Date:	9/4/96											
Dilution Water	0.00	0.0E+00	8.52	8.44	0.08							
Dilution Water	0.00	0.0E+00	8.57	8.37	0.20							
					0.14							
Seed Control	2.00	6.7E-03	8.56	8.02	0.54				81			
Seed Control	5.00	1.7E-02	8.48	7.31	1.17				70			
Seed Control	7.00	2.3E-02	8.46	6.65	1.81				78			
				Seed DO	0.51	0.04			76	6		
Glucose-Glutamic Acid	6.00	2.0E-02	8.44	3.94	4.50				200			
Glucose-Glutamic Acid	6.00	2.0E-02	8.42	3.99	4.43				196			
Dipropylene Glycol	0.100	3.3E-04	8.43	7.81	0.62		0.11	0.04	1,860		335	110
Dipropylene Glycol	0.100	3.3E-04	8.46	8.03	0.43		-0.08	0.04	1,290		-235	110
Dipropylene Glycol	0.100	3.3E-04	8.52	7.38	1.14		0.63	0.04	3,420		1,895	110
		3.3E-04			0.73	0.37	0.22	0.37	2,190	1,103	**665**	**1,108**
Dipropylene Glycol	0.200	6.7E-04	8.47	7.91	0.56		0.05	0.04	840		77	55
Dipropylene Glycol	0.200	6.7E-04	8.48	8.18	0.30		-0.21	0.04	450		-313	55
Dipropylene Glycol	0.200	6.7E-04	8.41	8.06	0.35		-0.16	0.04	525		-238	55
		6.7E-04			0.40	0.14	-0.11	0.14	605	207	**-158**	**214**
Dipropylene Glycol	0.500	1.7E-03	8.44	8.06	0.38		-0.13	0.04	228		-77	22
Dipropylene Glycol	0.500	1.7E-03	8.42	8.02	0.40		-0.11	0.04	240		-65	22
Dipropylene Glycol	0.500	1.7E-03	8.41	7.98	0.43		-0.08	0.04	258		-47	22
		1.7E-03			0.40	0.03	-0.11	0.04	242	15	**-63**	**27**
Dipropylene Glycol	1.000	3.3E-03	8.42	7.89	0.53		0.02	0.04	159		6	11
Dipropylene Glycol	1.000	3.3E-03	8.29	7.50	0.79		0.28	0.04	237		84	11
Dipropylene Glycol	1.000	3.3E-03	8.39	7.98	0.41		-0.10	0.04	123		-30	11
		3.3E-03			0.58	0.19	0.07	0.20	173	58	**20**	**59**

Dipropylene Glycol	2.000	6.7E-03	8.36	7.69	0.67		0.16	0.04	101		24	6
Dipropylene Glycol	2.000	6.7E-03	8.36	7.51	0.85		0.34	0.04	128		51	6
Dipropylene Glycol	2.000	6.7E-03	8.34	7.77	0.57		0.06	0.04	86		9	6
		6.7E-03			0.70	0.14	0.19	0.15	105	21	**28**	**22**

Completion Date:	9/9/96											
Dilution Water	0.00	0.0E+00	8.58	8.40	0.18							
Dilution Water	0.00	0.0E+00	8.59	8.42	0.17							
					0.18							
Seed Control	2.50	8.3E-03	8.53	7.97	0.56				67			
Seed Control	5.00	1.7E-02	8.44	7.25	1.19				71			
Seed Control	7.00	2.3E-02	8.37	6.15	2.22				95			
				Seed DO	**0.65**	0.13			**78**	**15**		
Glucose-Glutamic Acid	6.00	2.0E-02	8.30	4.10	4.20		3.64		210			
Glucose-Glutamic Acid	6.00	2.0E-02	8.31	4.27	4.04		3.48		202			

Dipropylene Glycol	0.002	6.7E-06	8.34	7.92	0.42		-0.23	0.13	63,000		-34,393	18,834
Dipropylene Glycol	0.002	6.7E-06	8.38	7.72	0.66		0.01	0.13	99,000		1,607	18,834
Dipropylene Glycol	0.002	6.7E-06	8.35	7.91	0.44		-0.21	0.13	66,000		-31,393	18,834
Dipropylene Glycol	0.002	6.7E-06	8.32	7.95	0.37		-0.28	0.13	55,500		-41,893	18,834
Dipropylene Glycol	0.002	6.7E-06	8.32	7.91	0.41		-0.24	0.13	61,500		-35,893	18,834
Dipropylene Glycol	0.002	6.7E-06	8.34	7.75	0.59		-0.06	0.13	88,500		-8,893	18,834
		6.7E-06			0.49	0.12	-0.16	0.18	74,100	18,693	**-23,293**	**26,536**

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	9/11/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Std Deviation	Corrected O2 Depletion	Correct O2 Std Dev	Partial BOD (mg/L)	Partial BOD Std Deviation	**Actual BOD (mg/L)**	**Actual BOD Std Deviation**
Dilution Water	0.00	0.0E+00	8.53	8.37	0.16							
Dilution Water	0.00	0.0E+00	8.50	8.39	0.11							
					0.14							
Seed Control	2.00	6.7E-03	8.49	7.90	0.59				89			
Seed Control	5.00	1.7E-02	8.45	7.74	0.71				43			
Seed Control	7.00	2.3E-02	8.42	7.57	0.85				36			
				Seed DO	0.37	0.19			56	28		
Glucose-Glutamic Acid	6.00	2.0E-02	8.39	4.04	4.35		3.98		199			
Glucose-Glutamic Acid	6.00	2.0E-02	8.38	4.30	4.08		3.71		185			
DiEGME	0.002	6.7E-06	8.43	7.60	0.83		0.46	0.19	124,500		68,657	28,450
DiEGME	0.002	6.7E-06	8.34	7.38	0.96		0.59	0.19	144,000		88,157	28,450
DiEGME	0.002	6.7E-06	8.34	7.58	0.76		0.39	0.19	114,000		58,157	28,450
		6.7E-06			0.85	0.10	0.48	0.22	127,500	15,223	**71,657**	**32,267**
DiEGME	0.004	1.3E-05	8.35	7.36	0.99		0.62	0.19	74,250		46,329	14,225
DiEGME	0.004	1.3E-05	8.31	6.94	1.37		1.00	0.19	102,750		74,829	14,225
DiEGME	0.004	1.3E-05	8.33	7.05	1.28		0.91	0.19	96,000		68,079	14,225
		1.3E-05			1.21	0.20	0.84	0.27	91,000	14,893	**63,079**	**20,595**

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	9/20/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Std Deviation	Corrected O2 Depletion	Correct O2 Std Dev	Raw BOD (mg/L)	Raw BOD Std Deviation	**Actual BOD (mg/L)**	**Actual BOD Std Deviation**
Dilution Water	0.00	0.0E+00	8.36	8.30	0.06							
Dilution Water	0.00	0.0E+00	8.39	8.25	0.14							
					0.10							
Seed Control	3.00	1.0E-02	8.43	7.69	0.74				74			
Seed Control	6.00	2.0E-02	8.41	8.09	0.32				16			
Seed Control	9.00	3.0E-02	8.41	8.21	0.20				7			
				Seed DO	**0.32**	**0.36**			32	36		
Glucose-Glutamic Acid	6.00	2.0E-02	8.44	4.67	3.77		3.45		172			
Glucose-Glutamic Acid	6.00	2.0E-02	8.44	4.39	4.05		3.73		186			
GF	0.010	3.3E-05	8.43	8.15	0.28		-0.04	0.36	8,400		-1,267	10,944
GF	0.010	3.3E-05	8.46	8.05	0.41		0.09	0.36	12,300		2,633	10,944
GF	0.010	3.3E-05	8.43	8.03	0.40		0.08	0.36	12,000		2,333	10,944
		3.3E-05			**0.36**	**0.07**	**0.04**	**0.37**	**10,900**	**2,170**	**1,233**	**11,157**
GF	0.020	6.7E-05	8.43	7.95	0.48		0.16	0.36	7,200		2,367	5,472
GF	0.020	6.7E-05	8.43	8.18	0.25		-0.07	0.36	3,750		-1,083	5,472
GF	0.020	6.7E-05	8.42	7.85	0.57		0.25	0.36	8,550		3,717	5,472
		6.7E-05			**0.43**	**0.17**	**0.11**	**0.40**	**6,500**	**2,475**	**1,667**	**6,006**

Biochemical Oxygen Demand - Worksheet / Sample Data

Completion Date:	9/11/96											
Sample Description	Sample (ml)	Conc. (ml/ml)	Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	O2 Std Deviation	Corrected O2 Depletion	Correct O2 Std Dev	Raw BOD (mg/L)	Raw BOD Std Deviation	**Actual BOD (mg/L)**	**Actual BOD Std Deviation**
Dilution Water	0.00	0.0E+00	8.53	8.37	0.16							
Dilution Water	0.00	0.0E+00	8.50	8.39	0.11							
					0.14							
Seed Control	2.00	6.7E-03	8.30	7.80	0.50				75			
Seed Control	5.00	1.7E-02	8.26	7.66	0.60				36			
Seed Control	7.00	2.3E-02	8.21	7.55	0.66				28			
				Seed DO	**0.31**	0.17			**46**	25		
Glucose-Glutamic Acid	6.00	2.0E-02	8.23	4.13	4.10		3.79		190			
Glucose-Glutamic Acid	6.00	2.0E-02	8.23	4.06	4.17		3.86		193			
Dipropylene Glycol	0.002	6.7E-06	8.18	7.60	0.58		0.27	0.17	87,000		40,571	25,042
Dipropylene Glycol	0.002	6.7E-06	8.17	7.64	0.53		0.22	0.17	79,500		33,071	25,042
Dipropylene Glycol	0.002	6.7E-06	8.19	7.60	0.59		0.28	0.17	88,500		42,071	25,042
		6.7E-06			0.57	0.03	0.26	0.17	85,000	4,822	**38,571**	**25,502**
Dipropylene Glycol	0.004	1.3E-05	8.15	7.54	0.61		0.30	0.17	45,750		22,536	12,521
Dipropylene Glycol	0.004	1.3E-05	8.12	7.46	0.66		0.35	0.17	49,500		26,286	12,521
Dipropylene Glycol	0.004	1.3E-05	8.10	7.42	0.68		0.37	0.17	51,000		27,786	12,521
		1.3E-05			0.65	0.04	0.34	0.17	48,750	2,704	**25,536**	**12,810**

Inihibitory 5 -Day BOD worksheets

Date:	10/1/96

Dilution Water Check (less than 0.2 mg/L required)

Sample #	Bottle Number	Sample Description	Sample (ml)	Sample2 (ml)		Initial DO (mg/L)	Final DO (mg/L)	O2 Depletion (mg/L)	BOD_1 (mg/L)	BOD_2 (mg/L)
1	16	Dilution Water	0.00			8.02	7.91	0.11		
2	51	Dilution Water	0.00			8.03	7.83	0.20		

Seed Controls (0.6 to 1.0 mg/L required)

3	20P	Seed Control	2.50			7.96	6.98	0.98	118
4	38	Seed Control	2.50			7.95	6.86	1.09	131
5	216	Seed Control	2.50			7.91	7.02	0.89	107
							Seed DO	**0.99**	

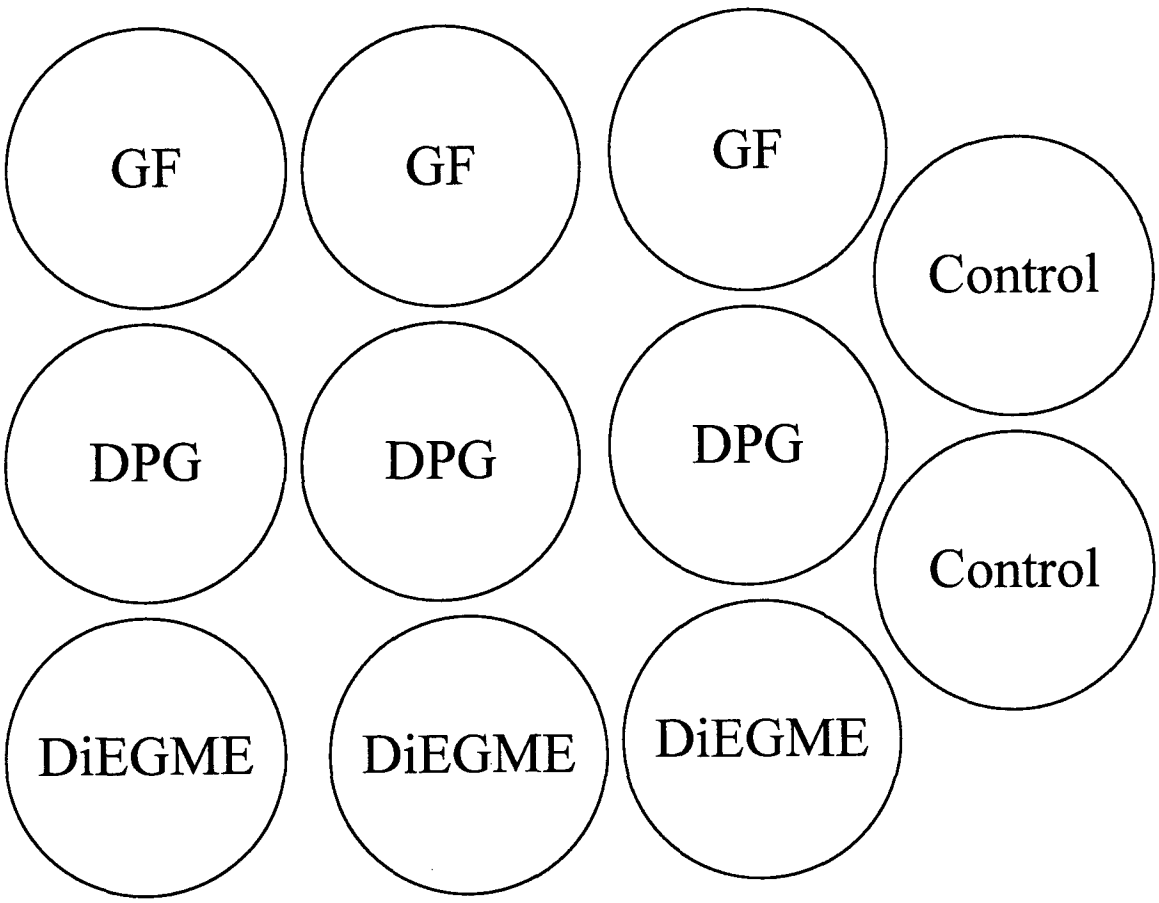
Glucose/Glutamic Acid Checks (198 mg/L +/- 30.5 mg/L required)

6	8P	Glucose-Glutamic Acid	6.00			7.89	3.38	4.51	176
7	203	Glucose-Glutamic Acid	6.00			7.83	3.30	4.53	177
7b	17	Glucose-Glutamic Acid	6.00			7.87	3.32	4.55	178
		Average							177
		Standard Deviation							1

8	212	DiEGME	0.002	0.000		7.80	4.55	3.25	339500	
9	37	DiEGME	0.002	0.000		7.80	5.00	2.80	272000	
10	18	DiEGME	0.002	0.000		7.78	4.94	2.84	278000	
11	4	DiEGME	0.002	0.000		7.78	5.02	2.76	266000	
12	5	DiEGME	0.002	0.000		7.82	4.80	3.02	305000	
13	204	DiEGME	0.002	0.000		7.80	4.99	2.81	273500	
		Average						2.91	289000	
		Standard Deviation						0.19	28233	
14	207	DiEGME+G/G	0.002	2.000		7.78	3.00	4.78	289000	280
15	230	DiEGME+G/G	0.002	2.000		7.81	3.96	3.85	289000	141
16	13	DiEGME+G/G	0.002	2.000		7.83	3.00	4.83	289000	288
		Average							289000	236
		Standard Deviation							0	87
17	208	DPG+G/G	0.002	6.000		7.81	3.19	4.62	-	182
18	17P	DPG+G/G	0.002	6.000		7.80	3.39	4.41	-	171
19	222	DPG+G/G	0.002	6.000		7.81	3.14	4.67	-	184
		Average								179
		Standard Deviation								7
20	200	GF+G/G	0.002	6.000		7.84	2.96	4.88	-	195
21	8	GF+G/G	0.002	6.000		7.84	3.30	4.54	-	178
22	213	GF+G/G	0.002	6.000		7.83	2.96	4.87	-	194
		Average								189
		Standard Deviation								10

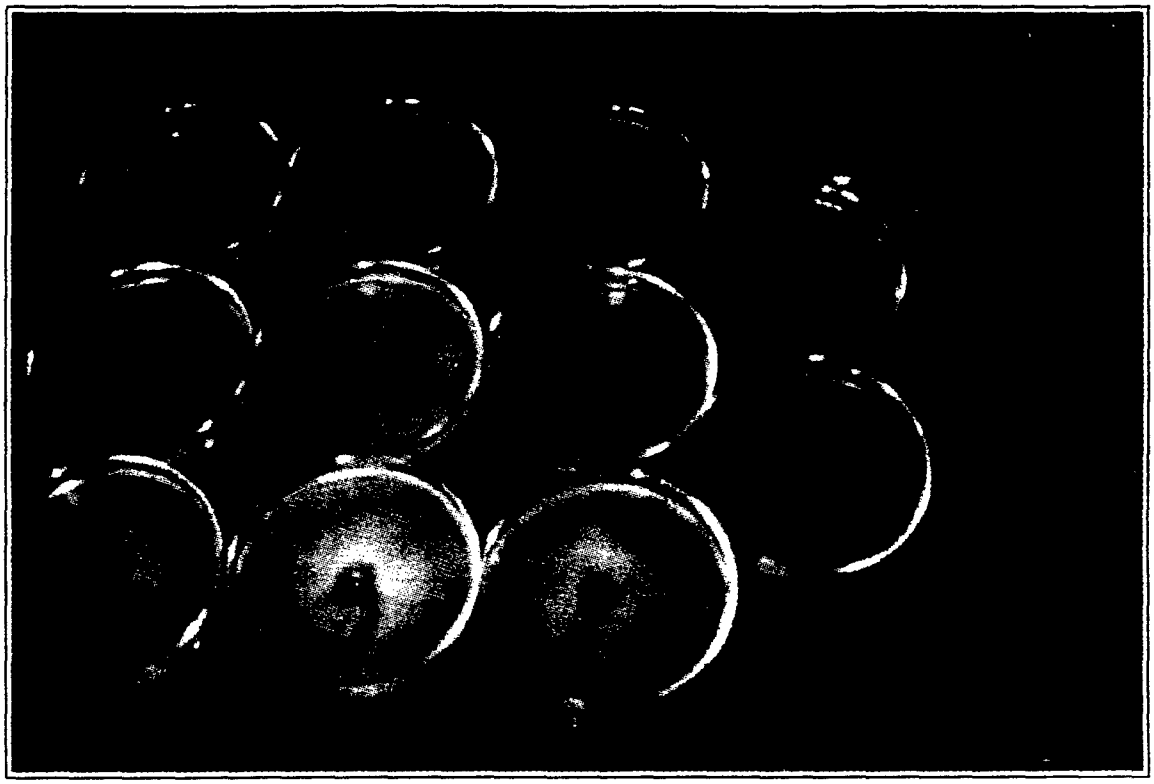
Appendix D

Agar Diffusion Toxicity Test Key



Appendix D

Agar Diffusion Toxicity Test



Vita

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13. ABSTRACT *(Maximum 200 words)*

The biodegradation characteristics of three fuel system icing inhibitors (FSII) were evaluated. FSII are jet fuel additives that partition into water readily and are present in the water drained from storage tank bottoms in concentrations approaching 40%. These concentrations raise concerns as to the disposal and handling of these wastes. The current FSII, DiEGME was evaluated along with two new candidates, dipropylene glycol and glycerol formal. DiEGME appeared to be moderately but not completely biodegradable. It is likely that much of it would be removed in a wastewater treatment plant. Dipropylene glycol only showed signs of degradation after more than three weeks at which point it degraded moderately well. The third FSII, glycerol formal did not show any signs of biodegradability during the five week period of testing. Preliminary toxicity and inhibitory tests were carried out for these chemicals at high and low concentrations. DiEGME appeared to be most toxic to microorganisms at high concentrations, dipropylene glycol show moderate toxicity, and glycerol formal showed little. At low concentrations, none of the chemicals appeared to inhibit the activity of microorganisms.

14. SUBJECT TERMS

biochemical oxygen demand, BOD, biodegradation, fuel system icing inhibitors, tank bottoms, DiEGME, diethylene glycol monomethyl ether, glycerol formal, dipropylene glycol

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